Improving the outcomes for high-risk neuroblastoma through the optimisation of radiotherapeutic techniques

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Declaration

I, Jennifer Elizabeth Gains confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed _____ Date _____

Abstract

Neuroblastoma is a childhood cancer with highly variable clinical behaviour and outcomes. The long-term survival rate for high-risk neuroblastoma remains poor and new therapeutic advances and optimisation of existing therapies is therefore required.

Both external beam radiotherapy and molecular radiotherapy have a significant role to play in the multi-modality treatment of high-risk disease. This collection of work examines ways in which the outcome for neuroblastoma may be improved through the introduction of new and enhancement of existing radiotherapeutic techniques.

^{131}I-*meta*-Iodobenzylguanidine molecular radiotherapy has been used in the treatment of neuroblastoma since the mid 1980's. Despite this, its role and efficacy remain undefined. A systematic review of ^{131}I-mIBG therapy in neuroblastoma is therefore presented.

Radiolabelled somatostatin analogues target a distinct and separate molecular target on neuroblastoma cells to the noradrenaline transporter targeted by ^{131}I-mIBG. This study reports on the use of radiolabelled somatostatin analogues for the imaging and therapy of patients with high-risk neuroblastoma. The expression of the two different molecular targets by immunohistochemistry for the noradrenaline transporter molecule and somatostatin receptor type-2 in archived neuroblastoma

tumour samples is also explored. The radiation doses received by comforters and carers providing necessary support to children undergoing molecular radiotherapy over a 10 year period is presented.

The gold standard imaging modality for response assessment in neuroblastoma is ^{123}I-mIBG scinitgraphy. The role of other functional imaging techniques such as ^{18}F-FDG PET/CT remains undefined. This study will look to see if ^{18}F-FDG PET/CT can give additional information with regards to response assessment.

External beam radiotherapy is standardly delivered using conventional anterior and posterior parallel opposed beams and this can result in a compromise on target volume coverage to stay within the tolerance of normal tissues. The use of an Intensity Modulated Arc Therapy technique to improve target volume coverage is examined.

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List of Abbreviations

AA	Administered Activities
AACIS	Automated Childhood Cancer Information System
ABMT	Autologous Bone Marrow Transplantation
ALARA	As low as reasonably achievable
ALK	Anaplastic lymphoma kinase
ASR	Age-standardised incidence rate
CI	Confidence Interval
CI	Conformity Index
CNS	Central Nervous System
COG	Children's Oncology Group
CR	Complete Response
CSI	Craniospinal radiotherapy
CT	Computed Tomography
CTV	Clinical Target Volume
EFS	Event Free Survival
^{18}F-FDG PET/CT	flurodeoxyglucose positron emission tomography/ computed tomography
FFPE	Formalin fixed paraffin embedded
^{68}Ga-DOTATATE PET/CT	^{68}Gallium DOTATATE positron emission tomography/ computed tomography
GPOH	German Paediatric Oncology Group
GTV	Gross Tumour Volume
HI	Homogeneity Index

IDRF	Image Defined Risk Factors
INRC	International Neuroblastoma Response Criteria
INRG	International Neuroblastoma Risk Group
INRGSS	International Neuroblastoma Risk Group Staging System
INPC	International Neuroblastoma Pathologic Classification
INSS	International Neuroblastoma Staging System
IMAT	Intensity Modulated Arc Therapy
IMRT	Intensity Modulated Radiotherapy
LuDO	Lutetium DOTATATE
MATIN	mIBG and Topotecan in neuroblastoma
mIBG	*meta*-iodobenzylguanidine
MIP	maximum intensity projection
MKI	Mitosis-karyorrhexis index
MRD	Minimal Residual Disease
MRI	Magnetic Resonance Imaging
NAT	Noradrenaline Transporter Molecule
NPID	Non-PTV Integral Dose
OMS	Opsoclonus Myoclonus Syndrome
OR	Odds Ratio
OS	Overall Survival
PBSCT	Peripheral Blood Stem Cell Tranplant
PERCIST	PET Response Criteria in Solid Tumours
PD	Progressive Disease

PET	Positron Emission Tomography
PFS	Progression Free Survival
PR	Partial Response
PRRT	Peptide Receptor Radionuclide Therapy
PTV	Planning Target Volume
RCT	Randomised Control Trial
SCC	Spinal Cord Compression
SIOPEN	International Society of Paediatric Oncology (Europe) Neuroblastoma
SPECT	Single photon emission tomography
SSSTR	Somatostatin receptor
TBI	Total Body Irradiation
USS	Ultrasound scan
VGPR	Very Good Partial Response
VIP	Vasoactive Intestinal Peptide

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Chapter 1

Introduction

1.1 History

Neuroblastoma was first described as a 'glioma' in the abdomen of a child, by a German physician, Rudolf Virchow, in 1864. Between 1901 and 1910 three important papers were published and these played an important role in defining the disease, neuroblastoma (*Rothenberg 2009*).

Two of the physicians, William Pepper and Robert Hutchinson, both published papers describing the pattern of spread of neuroblastoma, although they both thought they were describing a 'sarcoma'. In 1901, Pepper described the distinctive pattern in infants, of adrenal tumours and massive liver involvement, which we now know as stage MS disease (*Pepper 1901*). Robert Hutchinson, in 1907, reported a series of older children with disease spread to the orbit and skull as the first sign of their disease, which we now recognise as stage M (*Hutchinson 1907*).

James Homer Wright, in 1910, was the first person to understand and describe the tumour arising from primitive neural cells and to name the disease "*neuroblastoma"*. He recognised that adrenal tumours in children, with neural fibrils and bundles of cells (rosettes), resembled the morphology of the foetal adrenal (*Wright 1910*). ''Homer Wright rosettes'' are still used in the diagnosis of neuroblastoma today.

1.2 Embryology

Neuroblastoma is an embryonal tumour derived from the sympathetic nervous system. The embryonal structure that gives rise to the sympathetic nervous system is the neural crest, located on both sides of the developing spinal cord. In the developing embryo, neuroblasts migrate along the neuraxis and populate sympathetic ganglia in the adrenal medulla and other sites. The cell of origin for a neuroblastoma is thought to be a developing precursor cell derived form neural-crest tissues. The embryological development explains why neuroblastomas can arise at primary sites distributed along the length of the body from the neck to the pelvis, usually in a paravertebral location.

1.3 Epidemiology

Neuroblastoma accounts for about 7% of all childhood cancers and is the commonest extra-cranial solid tumour of childhood. The Automated Childhood Cancer Information System (AACIS) is a framework set up to collect and present data on childhood cancer through collaboration through several European countries. A report by the ACCIS in the European Journal of Cancer in 2006 reported incidence and survival for 19 countries from 59 cancer registries in Europe. The age-standardised incidence rate (ASR) of neuroblastoma in Europe between 1988 and

1997 was 10.9 cases per million children and was highest in infants (52.6). The incidence of neuroblastoma was highest in the first year of life, declined afterwards and was rare beyond 10 years of age. (*Spix 2006*)

The average 5 year survival from neuroblastoma from 1988-1997 in Europe as a whole was 59%. There was a significant effect of age on survival with infants having a 5 year survival of 84%, those aged between 1 -4 years 47% and the 10-14 year old age group having a 5 year survival of 38%. It is noted that data on the stage of disease was not collected for the ACCIS report. (*Spix 2006)*

There were also significant regional differences in survival across Europe with all ages combined – 47% in the East, 49% in the British Isles, 62% in the South, 67% in the West and 57% in the North (*Spix 2006*).

There was evidence from the same report that survival for neuroblastoma has been improving over time. A significant increase in 5-year survival was seen from the late 1970's (21%) to the mid 1990's (45%) ($p<0.001$) (*Spix 2006*).

With regards to the UK alone, there were only on average 92 patients aged between 0-14 years diagnosed annually in the UK between 2001 and 2007 (*National Registry 2010*). This means that there are too few patients for UK-only based randomised control trials and international collaboration is essential.

Although neuroblastoma accounts for approximately 7% of all cases of malignancy diagnosed in children under the age of 16 years, it causes a disproportionate number of deaths within this age group (approximately 15%).

The aetiology of neuroblastoma remains poorly understood. No definitive association has been found between maternal or paternal exposures or occupations, or other potential factors (*Heck 2009*). Genetic factors are likely to play a major role as discussed below.

1.4 Genetics

1.4.1 Familial Neuroblastoma

A family history of neuroblastoma is found in approximately 1-2% of cases. There is usually an autosomal dominant pattern of inheritance with incomplete penetrance.

The majority of hereditary neuroblastomas can be accounted for by mutations in the tyrosine kinase domain of the anaplastic lymphoma kinase (ALK) oncogene (*Mosse 2008*). Germ-line mutations encode for single base substitutions in key regions of the kinase domain resulting in constitutive activation of ALK. Somatic mutations of ALK have also been found in 5 to 15% of neuroblastomas (*Janoueix-Lerosey 2008, George 2008*).

The other germ line mutation identified in familial neuroblastoma is *PHOX2B.* (*Trochet 2004, Mosse 2004*). Two neural crest developmental disorders are associated with PHOX2B mutations – Hirschsprung's disease and Ondine's curse. Somatic mutations in PHOX2B are rare.

1.4.2 Sporadic Neuroblastoma

The vast majority of neuroblastomas are sporadic. The most common cytogenetic aberrations include *MYCN* amplification, and segmental chromosomal abnormalities such as deletions of chromosome 1p, 3p, 11q and gain of 1q, 2p and 17q. The prognostic significance of these is discussed in the risk stratification section below (section 1.8.3).

Neuroblastoma patients are risk stratified into prognostic groups and these are used to guide treatment choice and indicate prognosis. The groups are low, intermediate and high risk and are stratified by disease stage, age, *MYCN* amplification, histopathology, and allelic loss of chromosomal regions at 1p and 11q and DNA ploidy. Despite this, there are still some low and intermediate risk patients who still die from their disease and some low risk patients who would potentially spontaneously regress and could be spared treatment. There is increasing evidence that many different biological subgroups of neuroblastoma exist and differ in their gene expression patterns. This will hopefully, in the future, help us to improve classification of neuroblastoma patients (*Oberthuer 2010*). A retrospective study by

the SIOPEN/COG/GPOH groups used a 59-gene expression signature and found it to be an accurate predictor of outcome in patients with neuroblastoma (*Vermeulen 2009*). This technology will now require evaluation in large prospective clinical trials.

1.5 Screening

Neuroblastoma was considered to be a good candidate disease for a screening programme for several reasons (*Morris 2002*):

1. Children diagnosed under the age of 18 months are known to have a better prognosis and therefore diagnosing infants early with screening was hoped to have an impact on outcome
2. Children presenting with advanced disease have a worse prognosis
3. There was a simple screening test available – measuring catecholamine metabolites in the urine, sampled by blotting wet nappies with filter paper. This screening test had been found to detect 90% of neuroblastomas in children screened (*Tuchman 1987*).

Mass screening was adopted in Japan and many screening studies were performed in the early 1990's mostly in Canada, Germany, France, Austria, Italy, United States and Australia. The two studies measuring mortality from Canada and Germany were reported in the New England Journal of Medicine in 2002 and both showed that screening infants for neuroblastoma did not reduce mortality from the disease

(*Woods 2002, Schilling 2002*). The studies showed that screening led to 'over-diagnosis' and therefore 'over-treatment' by identifying tumours that would have spontaneously regressed. There is therefore, no national screening programme for neuroblastoma in the UK.

1.6 Clinical Presentation

The clinical manifestations of neuroblastoma are variable depending on the site of the primary tumour and whether metastatic disease is present or not. Symptoms of neuroblastomas can be divided into those caused by the primary tumour, those cause by the presence of metastases of those caused by the co-existence of a paraneoplastic syndrome.

1.6.1 Primary Tumours

Neuroblastoma is an embryonal tumour derived from cells of neural crest origin, which form the sympathetic nervous system. Neuroblastomas most frequently arise in the adrenal medulla but can be found in other sites related to the sympathetic chain or paraganglia. Approximately 70% of primaries are found in the abdomen (divided in to supra-renal and retroperitoneal locations), 25% in the thorax and 5% in the pelvis.

A child with a hard, fixed, abdominal mass causing mild abdominal discomfort is a frequent presentation of a primary tumour. If the tumour is compressing renal vessels, hypertension may be present. Primaries presenting in the cervical region can be mistaken for cervical lymph nodes and primaries in the cervical or thoracic region can present with a Horner's syndrome.

Primaries in the thoracic, cervical, abdominal or pelvic region can extend into the neural foramina and compress nerve roots or the spinal cord (dumb-bell tumours). Up to 5% of newly diagnosed patients can have neurological signs related to cord impingement or compression (*de Bernardi 2005*) (see section 1.12.1).

1.6.2 Metastatic Disease

The commonest sites for metastases in neuroblastoma are bone, bone marrow, liver and lymph nodes. Bone metastases are often painful resulting in an unwell, irritable child at presentation. A limp may be present due to bone metastases in the lower limbs or the pelvis. If there is bone marrow involvement this usually presents with anaemia and later thrombocytopenia. Orbital metastatic masses can cause proptosis.

Infants with stage MS disease can present with hugely enlarged livers and can cause significant respiratory distress. These patients can also have non-tender, bluish in colour, subcutaneous nodules.

1.6.3 Paraneoplastic Syndromes

Hypertension, tachycardia and sweating are less common in neuroblastomas than in phaeochromocytomas.

Opsoclonus myoclonus syndrome is a rare presentation of neuroblastoma and consists of myoclonic, irregular, jerking, random eye movements and cerebellar ataxia. It occurs in a reported 2-4% of children with neuroblastoma. The syndrome is usually associated with good-prognosis disease but 70-80% of children will be left with long-term neurological deficits (see section 1.12.3).

Kerner-Morrison syndrome is a syndrome caused by secretion of vasoactive intestinal peptide (VIP) and causes intractable, watery diarrhoea. It usually resolves after removal of the tumour (*Kaplan 1980*).

1.7 Disease Assessment

Being able to accurately stage patients with neuroblastoma is essential. The stage of disease not only has prognostic implications but also guides the treatment strategy for individual patients. Having an International staging and classification system (as discussed in section 1.8) facilitates the standardisation of risk-based clinical trials internationally. Guidelines by the International Neuroblastoma Risk Group (INRG) on the imaging and staging of neuroblastic tumours have recently been published (*Brisse 2011*).

A definitive diagnosis of neuroblastoma is normally made by biopsy of an affected site but diagnosis is also considerably helped by other investigations listed below and these are also required for the full staging of the disease.

1.7.1 Primary Tumour Assessment

Children who present with an abdominal mass are usually initially investigated with an ultrasound scan (USS) as it is a non-invasive procedure and is widely available. It is often able to guide the radiologist to the best area to perform a biopsy or to image next with a Magnetic Resonance Imaging (MRI) or computed tomography (CT) scan. USS does have limitations and the INRG recommends an MRI or CT as well for accurate staging of the primary tumour and the identification of Imaged Defined Risk Factors (IDRF's) (*Brisse 2011*).

With regards to CT versus MRI, nowadays there may be not much difference in image quality between the two modalities. A CT scan has the advantage that it: is readily available in most centres; is fast to acquire the images; is easily reproducible between centres; can in many cases be done without an anaesthetic as it is quicker and less claustrophobic than an MRI; can be good for surgical planning and assessing vascular invasion. But, CT does involve a dose of radiation to the child, and, often CTs may be done without contrast or performed on adult protocols resulting in poorer image quality. There can also be poor soft tissue definition with CT in children. CT is needed for full evaluation of lung parenchyma to demonstrate pulmonary metastases, although these are uncommon.

MRI scans do not involve a radiation exposure for the child but there is often a lack of reproducibility of scans between centres and scans take a lot longer to acquire which normally involve a general anaesthetic in babies and younger children. MRI is particularly useful for assessing intra-spinal masses, lymph node involvement and marrow invasion. Whole body MRI could be useful for assessing metastatic disease in mIBG negative patients.

1.7.2 Metastatic Disease Assessment

Functional imaging with ^{123}I-mIBG is the gold standard imaging modality for diagnosis, staging and response assessment of metastatic neuroblastoma. The majority of children with neuroblastoma, approximately 90%, take up mIBG. mIBG is a guanethidine derivative and can be labelled with ^{123}I for imaging or ^{131}I for therapy. There is no physiological uptake of mIBG in bone or bone marrow and therefore mIBG scintigraphy is an accurate method for detecting metastases in cortical bone or bone marrow.

^{123}I-mIBG is preferred to ^{131}I-mIBG for diagnostic imaging as it has a shorter half-life therefore reducing the radiation exposure. The γ emissions are also of a lower energy contributing to a lower radiation exposure and making them better suited to γ cameras. ^{123}I-mIBG scintigraphy has a high sensitivity (90%) and specificity (100%) in neuroblastoma (*Lumbroso 1988, Jacobs 1990*). It is essential however, that mIBG scintigraphy is performed with standardised procedures (*Bombardieri 2010*) and reported in a standardised way to improve consistencies in reporting between different institutions. The INRG task force has recently published guidelines on this (*Matthay 2010*).

Although ^{123}I-mIBG scintigraphy is the current gold standard imaging modality for high-risk neuroblastoma metastatic disease, it does has limited spatial resolution and small amounts of bone marrow disease can go undetected by this method. Bilateral

bone marrow aspirations and trephines are still therefore essential for detecting bone marrow disease.

The use of mIBG SPECT (single photon emission tomography) can improve the visualisation of small foci of neuroblastoma that can be difficult to detect on planar scintigraphy and is becoming more widely available. SPECT can also be fused with CT images to improve the anatomical localisation. (*Gelfrand 1994, Rozovsky 2008*).

Historically, for patients with mIBG negative disease, a ^{99m}Tc MDP bone scan has been recommended. These scans are often difficult to interpret in children with growing bones and have a lower specificity and sensitivity compared to ^{123}I-mIBG scintigraphy.

The role of ^{124}I-mIBG PET/CT is currently under investigation and could potentially improve the spatial resolution and sensitivity of imaging with mIBG. It combines the advantage of the specificity and sensitivity of mIBG with the superior spatial and anatomical resolution of PET/CT. ^{124}I is cyclotron produced with a 4.2 day half-life. The disadvantage is that ^{124}I also produces significant high-energy gamma emissions, which makes image acquisition more complicated.

Several other functional imaging modalities have emerged in recent years including ^{18}F-FDG PET/CT, ^{18}F-DOPA PET/CT and ^{68}Ga-DOTATATE PET/CT (*Sharp 2009, Piccardo 2012, Kroiss 2011*). It is still unclear what their role will be and these will be discussed in detail in Chapter 5.

Often patients with aggressive high-risk neuroblastoma will have dissemination of disease to their bone marrow. Accurate detection of bone marrow disease is therefore essential for accurate staging and treatment stratification. The INRGSS states that bone marrow involvement should be assessed by two aspirates and two biopsies from bilateral sites (*Monclair 2008*). This should be performed by the cytological screening of bone marrow aspirates and the histological assessment of bone marrow biopsies. The INRGSS defines cut off of bone marrow infiltration of >10% tumour cells as the level to be able to distinguish stage M from stage MS disease.

The INRG Task Force has published and defined standardised procedures for the determination of minimal residual disease (MRD) by immunocytochemistry and quantitative reverse transcriptase-polymerase chain reaction. The INRG standard operating procedures define methods for evaluating and processing peripheral blood, bone marrow and peripheral blood stem cell harvest. These should allow standardisation of procedures and facilitate their use in clinical trials on a multi-centre and international basis (*Beiske 2009*). Although MRD is used clinically in the management of Acute Lymphoblastic Leukaemia, the measurement of MRD in neuroblastoma remains experimental and will require evaluation in prospective clinical trials.

1.8 Risk Stratification

Neuroblastoma is highly heterogeneous in its clinical behaviour and can range from the near benign, with spontaneous regression in some cases, to highly aggressive disease which is fatal, despite the use of intensive multi-modality therapy. It is essential therefore that neuroblastoma patients are adequately staged and risk stratified depending on prognostic variables. Risk stratification should enable targeted and tailored therapy for patients with the aim of improving outcomes for high-risk patients and reducing the toxicity of treatment for those who require less intensive therapies.

The International Neuroblastoma Risk Group (INRG) was formed in 2004 when four cooperative groups encompassing Australia, Europe, Japan and North America agreed to pool their information (*Cohn 2009*). A database of thousands of children diagnosed with neuroblastoma was formed and the resulting publications have had a large impact on the management of neuroblastoma around the world.

The INRG Task Force looked at over 8000 patients with neuroblastoma from North America, Australia, Europe and Japan and examined the statistical and clinical significance of 13 potential prognostic factors (*Cohn 2009*). The stage of neuroblastoma, age, histologic category, grade of tumour differentiation, MYCN oncogene status, chromosome 11q status and DNA ploidy were all highly statistically significant for predicting outcome.

These prognostic variables formed the foundation for the International Neuroblastoma Risk Group (INRG) Consensus Pre treatment Classification (*Cohn 2009*) and each of these prognostic variables is discussed in more detail below.

1.8.1 Stage

Staging systems for neuroblastoma have evolved over time. The first staging system used for neuroblastoma was the Evans classification proposed in 1971 (*Evans 1971*). Following on from this, there were several different staging systems in use around the world, which made it very difficult to compare clinical trials and results from around the world. In a rare disease like neuroblastoma, international collaboration and comparison of results is an essential step in improving outcomes.

In 1986, an international meeting was held to establish a consensus on the criteria for diagnosis, staging and response assessment of neuroblastoma. The staging system proposed was the International Neuroblastoma Staging System (INSS). This was originally published in 1988 (*Brodeur 1988*) and subsequently revised in 1993 (*Brodeur 1993*). The INSS was based on the post-surgical assessment of disease and the details are shown in Figure (*Brodeur 1993*).

Table 1.1

The International Neuroblastoma Staging System (INSS) (adapted from *Brodeur 1993*)

Stage	Definition
1	Localised tumour with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nosed negative for tumour microscopically (nodes attached to and removed with the primary tumour may be positive)
2A	Localised tumour with incomplete gross excision; representative ipsilateral non-adherent lymph nodes negative for tumour microscopically
2B	Localised tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. Enlarged contralateral lymph nodes must be negative microscopically.
3	Unresectable unilateral tumour infiltrating across the midline, with or without regional lymph node involvement; or localised unilateral tumour with contralateral regional lymph node involvement; or midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement
4	Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and /or other organs (except as defined for 4S)
4S	Localized primary tumour (as defined for stage 1,2A or 2B) with dissemination limited to skin, liver and/or bone marrow (limited to infants <1 year of age)

In recent years, a new staging system has been developed by the INRG. This new staging system enables the stratification of patients at diagnosis rather than post-surgery and therefore removes the variation in the extent of surgery or the variation in definitions of operability. The International Neuroblastoma Risk Group Staging System (INRGSS) was published in 2009 and classifies patients at diagnosis and before treatment into one of four groups (see Figure 1.2) (*Monclair 2009*). Allocation to a particular stage is based on the local extent of the primary tumour and the absence or presence of metastatic disease.

It is important to have an understanding of the both of the staging systems as much of the published evidence uses the old INSS staging system.

Table 1.2

The International Neuroblastoma Risk Group Staging System (INRGSS) (adapted from *Monclair 2009)*

Stage	Description
L1	Localized tumour not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment
L2	Loco regional tumour with presence of one or more image-defined risk factors
M	Distant metastatic disease (except stage MS)
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver and/or bone marrow

Within the INRGSS, the primary tumour is assessed using USS, MRI and/or CT for the absence or presence of image defined risk factors (IDRF's). The IDRF's are surrogates for surgical risk. They have been shown to predict for the likelihood of surgical complications and are able to predict the likelihood of a complete surgical resection of the primary tumour (see Figure 1.3) (*Simon 2008, De Bernardi 2008*).

^{123}I-mIBG is the gold-standard imaging modality for evaluating metastatic disease but this is supplemented with bone marrow disease evaluation (bone marrow aspirates and trephines).

Patients with distant metastases are defined as stage M unless they meet the strict criteria for stage MS (corresponds to the INSS stage 4S disease).

Table 1.3

The Image-Defined Risk Factors in Neuroblastic Tumours (adapted from *Monclair 2009).*

Ipsilateral tumour extension within two body compartments • Neck-chest, chest abdomen, abdomen-pelvis
Neck • Tumour encasing carotid and/or vertebral artery and/or internal jugular vein • Tumour extending to the base of skull • Tumour compressing the trachea
Cervico-thoracic junction • Tumour encasing brachial plexus roots • Tumour encasing subclavian vessels and/or vertebral and/or carotid artery • Tumour encasing the trachea
Thorax • Tumour encasing the aorta and/or major branches • Tumour compressing the trachea and/or principal bronchi • Lower mediastinal tumour, infiltrating the costo-vertebral junction between T9 and T12
Thoraco-abdominal • Tumour encasing the aorta and/or vena cava
Abdomen/pelvis • Tumour infiltrating the porta heaptis and/or the hepatoduodenal ligment • Tumour encasing branches of the superior mesenteric artery at the mesenteric root • Tumour encasing the origin of the coeliac axis, and/or of the superior mesenteric artery • Tumour invading one or both renal pedicles • Tumour encasing the aorta and/ or vena cava • Tumour encasing the iliac vessels • Pelvic tumour crossing the sciatic notch
Intraspinal tumour whatever the location provided that: • More than one third of the spinal canal in the axial plane is invaded and/or • the perimedullary leptomeningeal spaces are not visible and/or • the spinal signal is abnormal
Infiltration of adjacent organs/ structures • Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, and mesentery
Conditions to be recorded, but not considered IDRF's • Multifocal primary tumours • Pleural effusion, with or without malignant cells • Ascites, with or without malignant cells

The stage of disease is highly predictive of outcome in neuroblastoma. Those with metastatic disease (part from stage MS) have a poorer outcome. Within the INRG patients evaluated – 5 year EFS was 35% for INSS stage 4 disease compared to 83% for stage 1,2,3 or 4S disease ($p<0.0001$). 5 Year OS was 42% for stage 4 compared to 91% for stage 1,2,3, and 4S (*Cohn 2009*).

1.8.2 Age

Age is a powerful prognostic factor and traditionally an age of 1 year was used as the cut off for prognostic grouping. A report and data analysis by *London et al*. on behalf of the INRG has shown strong evidence for the age cut off to be 460 days (*London 2005*). Within the INRG classification 18 months has been adopted as the new binary cut off point (*Cohn 2009*). For babies less than 18 months of age and no MYCN amplification the prognosis is dramatically better than for older children.

With the raising of the cut off point to 18 months of age, greater numbers of children who require less intensive treatment and have a good outcome will have been potentially spared the late effects and toxicity of more intensive therapy.

1.8.3 Molecular Pathology

The molecular pathology of neuroblastoma is complex and many molecular markers with prognostic significance have been discovered. The most widely used is *MYCN* amplification but other molecular tumour markers are important. The INRG task force identified *MYCN* status, 11q allelic status and tumour-cell ploidy as important in predicting a poorer outcome (*Cohn 2009*).

Association of the amplification of the *MYCN* oncogene and a poor prognosis was first reported in 1985 (*Seeger 1985*). The amplification of *MYCN* has significance at all stages of disease. In low stage tumours the INRG found that patients with INSS stage 1 and 2 disease had a worse 5 year OS in those with MYCN amplification (72% v 98%) (*Bagatell 2009*). In stage 4 disease, *MYCN* amplification is also prognostically significant. Within the INRG classification, patients older than 547 days and with a serum ferritin of <92 and a *MYCN* amplified tumour had an overall survival of 27% versus 53% for those with non *MYCN* amplified tumours. For those patients with a ferritin level ≥92, overall survival was 22% if they had *MYCN* amplified tumours versus 30% if *MYCN* non-amplified (*Cohn 2009*).

MYCN amplification is uncommon in infants, occurring in approximately 10% of cases. It is however predictive of poor survival rates despite aggressive treatment amongst this age group (*Canete 2009*).

Attiyeh *et al.* found 11q aberration in 34% of tumours in over 900 neuroblastomas screened (*Attiyeh 2005*). 11q aberration correlates inversely with the presence of *MYCN* amplification, and is therefore considered a further significant biomarker in non-*MYCN* amplified disease. Deletion of 11q was associated with poorer patient outcomes in the INRG classification system (*Cohn 2009*). Within INRG task force analysis, allelic status was combined with tumour grade differentiation for prognostic purposes and risk stratification for patients aged over 18 months with *MYCN* non-amplified INSS stage 2 or 3 tumours. Undifferentiated histology and/or 11q aberration were prognostic for 5 year OS of 73%, compared with a 5 year OS of 100% in patients without either factor (*Cohn 2009*).

Within the INRG risk classification tumour DNA ploidy was statistically significant in patients younger than 18 months of age with stage 4 disease and *MYCN* non-amplified tumours (*Cohn 2009*). In low-stage, *MYCN* amplified disease, outcomes were significantly greater in those with hyperdiploid rather than diploid tumours (5 year OS: 94% vs. 54%) (*Bagatell 2009*).

Multiplex Ligation-dependent Probe Amplification (MLPA) is now being used to rapidly select patients with segmental chromosomal abnormalities (*Combaret 2012*). The MLPA is now available for routine laboratory testing and the presence or absence of segmental chromosomal abnormalities (SCA) is being incorporated into prospective clinical studies for example, the SIOPEN Low- and Intermediate-risk Neuroblastoma European Study (LINES).

1.8.4 Histology

Within the International Neuroblastoma Pathologic Classification (INPC), tumours are evaluated depending on the degree of neuroblastoma maturation, the amount of stromal development and the mitosis-karyorrhexis index of the cells (*Shimada 1999*).

Tumours are classified into four categories –

1 *Neuroblastoma* (Schwannian stroma-poor);

2 *Ganglioneuroblastoma, Intermixed* (Schwannian stroma-rich);

3 *Ganglioneuroma* (Schwannian stroma-dominant);

4 *Ganglioneuroblastoma, Nodular* (composite, Schwannian stroma-rich/stroma dominant and stroma-poor).

Tumours within the neuroblastoma group are further divided depending on

1. the degree of neuroblastic differentiation (undifferentiated, poorly differentiated, and differentiating)

and

2. by their mitosis-karyorrhexis index (low, intermediate or high).

The INPC histology system divides patients into favourable and unfavourable groups depending on the patient's age at diagnosis and histological features described above. Tumour histology is another well-established prognostic variable. Within the

INRG classification those with favourable histology had a 5 year overall survival of 95% compared to 49% for those with unfavourable histology (*Cohn 2009*). Within the INRG classification histological categories were analysed independently. A low or intermediate MKI was associated with a better outcome (5 year overall survival of 82%) compared to a high MKI (44%) (p<.0001). Those patients with differentiating tumours had better 5 year overall survival at 89% rather than 72% for undifferentiating tumours (p<.0001). Neuroblastoma, stroma-poor patients had a 5 year overall survival of 71%; ganglioneuroblastoma, intermixed, stroma-rich 96%; ganglioneuroma, stroma-dominant 79%; and ganglioneuroblastoma, nodular (composite) 68% (p<.0001). Each factor had independent prognostic value.

1.8.5 International Neuroblastoma Risk Group Classification

Combining INRGSS stage with age at diagnosis, histological category, grade of tumour differentiation, *MYCN* status, chromosome 11q status and DNA ploidy, the INRG classification algorithmically stratifies individuals to four pre-treatment groups of broadly homogenous risk banded by EFS at five years (*Cohn 2009*). Treatment can subsequently be adjusted accordingly, with intensity of therapy guided by risk assessment of the projected behaviour of the disease.

Patients with very low risk disease often undergo spontaneous regression of the tumour and more than 85% are event free at five years. Patients with low risk disease can require little or no cytotoxic chemotherapy. Those patients with

intermediate risk require multimodal treatment and those in the high risk group receive aggressive multimodal therapy to improve their poor outcome. Prognostic variables that can reliably guide risk-group stratification and eschew the potential late effects following aggressive treatment are highly desirable.

Table 1.4

The International Neuroblastoma Risk Group (INRG) Consensus, Pre-treatment Classification schema (adapted from *Cohn 2009)*.

INRG Stage	Age (months)	Histologic Category	Grade of tumour differentiation	*MYCN*	11q Aberration	Ploidy	Pre-Treatment Risk Group
L1/L2		GN Maturing; GNB Intermixed					Very Low
L1		Any, except GN maturing or GNB intermixed		NA			Very Low
				Amp			High
L2	< 18	Any, except GN maturing or GNB intermixed		NA	No		Low
					Yes		Intermediate
	≥ 18	GNB nodular ; neuroblastoma	Differentiating	NA	No		Low
					Yes		Intermediate
			Poorly differentiated or undifferentiated	NA			Intermediate
				Amp			High
M	<18			NA		Hyperdiploid	Low
	<12			NA		Diploid	Intermediate
	12 to <18			NA		Diploid	Intermediate
	<18			Amp			High
	≥18						High
MS					No		Very Low
				NA	Yes		High
				Amp			High

1.9 Treatment

As has been discussed above, patients with neuroblastoma are risk stratified depending on age, stage and molecular pathology. I will briefly discuss the management of low and intermediate risk neuroblastoma but will focus on high-risk disease as this is the main area where radiotherapeutic modalities are used.

1.9.1 Low and Intermediate Risk Disease

As the EFS and OS for low- and intermediate- risk neuroblastoma have increased, the focus has been on reducing treatment intensity with the aim of minimizing the treatment burden, toxicity and long-term effects.

Infants with neuroblastoma without *MYCN* gene amplification generally have good outcomes. Those with unresectable disease and no *MYCN* amplification have had OS rates of 95–100% in the past with chemotherapy; however, there have been concerns over the long-term toxicity of such treatment. The INES 99.1 study evaluated the efficacy of low-dose chemotherapy in infants with non-metastatic and unresectable neuroblastoma without *MYCN* amplification with the aim of reducing total chemotherapy doses and avoiding anthracyclines without affecting outcomes for these infants. Low-dose chemotherapy without anthracyclines was effective in 62% of infants with unresectable, non *MYCN*-amplified tumors. This did not

compromise on OS (5-year OS: 99%) and may improve late toxicities for this group (*Rubie 2010*).

With regards to infants with disseminated neuroblastoma without *MYCN* amplification, most have a good outcome and De Bernardi *et al.* reported on two European trials focusing on reducing treatment intensity for this group. In trial 99.2, infants with either stage 4S disease or with stage 4 disease with a primary tumor crossing the midline or positive skeletal scintigraphy and no symptoms were observed. In trial 99.3, infants with overt metastases to the skeleton and/or lung and/or CNS were treated with a minimum of four chemotherapy courses. Both trials had OS rates of greater than 95% (*De Bernadi 2009*).

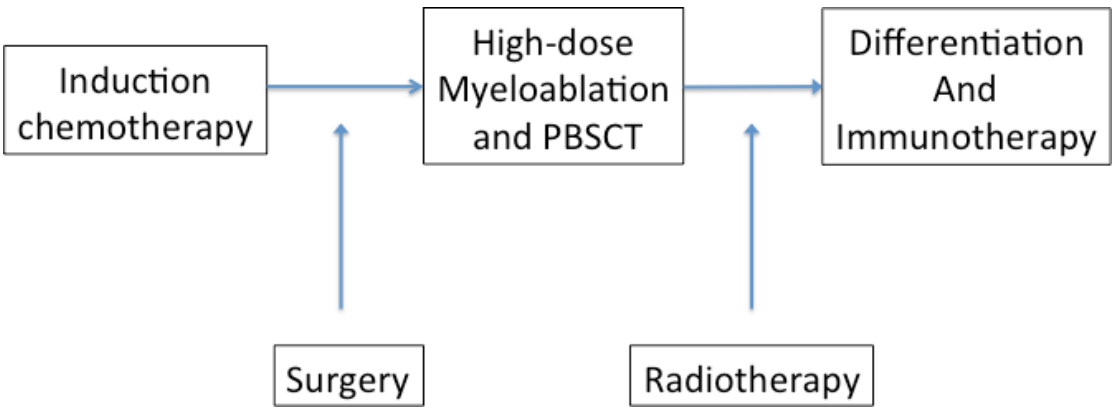
Within the European, LINES 2009 study (Low and Intermediate Risk Neuroblastoma European Study), the primary aim for the low risk patients is to minimise the amount of treatment for appropriate low–risk patients whilst maintaining their excellent long-term outcome. Patients requiring radiotherapy include those with INSS Stage 1 neuroblastoma with MYCN amplification and those with Stage L2, age > 18 months, MYCN non-amplified, poorly differentiated and undifferentiated neuroblastoma or poorly differentiated and undifferentiated ganglioneuroblastoma nodular.

1.9.2 High-risk disease

As discussed earlier it is not possible to perform clinical trials of high-risk disease treatment in the UK alone, as patient numbers are too small. Therefore, International collaboration for clinical trials is essential. International high-risk neuroblastoma trial protocols all involve a multimodality approach to treatment. I will focus on the current European high-risk neuroblastoma study – SIOPEN, but the on-going trials in the US, Germany and Japan all follow a similar multi-modality structure. This involves an induction chemotherapy regime, surgical resection of the primary tumour, high-dose myeloablative chemotherapy and peripheral blood stem cell support, external beam radiotherapy to the primary site and minimal residual disease therapy including immunotherapy. Each of these elements to therapy along with their evidence base will be discussed below.

Figure 1.1

The different components of the SIOPEN high-risk neuroblastoma trial



1.9.2.1 Induction Chemotherapy

Induction therapy is given with the aim of reducing the primary tumour size and hopefully facilitating a full surgical resection but also aims to increase the effectiveness of myeloablative therapy by reducing the metastatic burden.

A good response to initial induction therapy has been shown to correlate with a better outcome in high-risk neuroblastoma (*Kushner 1994*). Within the COG high-risk study patients with a CR or VGPR after induction had a significantly better outcome (*Matthay 2009*).

Unfortunately, approximately one third of high-risk patients do not respond to induction therapy and the outcome for these patients is very poor (*Ladenstein 2010*). Recent clinical trials have aimed to improve response rates by either using rapid induction schedules, increasing the dose intensity or the addition of new agents.

Most induction regimes include the following chemotherapy agents – cisplatin and/or carboplatin, cyclophosphamide, etoposide, vincristine and doxorubicin in some. The current SIOPEN high-risk trial uses rapid COJEC - a dose intensive, rapid induction regime. This regime was shown to improve the 3 year EFS when compared to a standard regime (OPEC) within the ENSG 5 trial (*Pearson 2008*).

The highest response rates to induction therapy (CR plus VGPR in 85%) have been reported by Kushner *et al* in the early 1990's using the N6 protocol and subsequently these results were replicated with the N7 protocol (*Kushner 1994, Cheung 2001*). The SIOPEN high-risk trial will now randomise between a modified N7 regime (N5) and rapid COJEC *(Valteau-Couanet 2005, Kohler 2007, Valteau-Couanet 2014*).

An alternative strategy to improve response rates to induction therapy is to use new agents with different cytotoxic mechanisms. Topotecan, a topoisomerase I inhibitor has been shown to be effective in induction regimes in early phase clinical trials (*Park 2011, De Ioris 2011*) and is now being incorporated into the COG phase II trial (COG-ANBL0532) as well as the current German high-risk neuroblastoma phase III trial.

Some groups have used a different approach and given molecular radiotherapy with ^{131}I-mIBG upfront as part of induction therapy (*de Kraker 2008, Mastrangelo 2011*).

1.9.2.2 Surgery

Surgical resection of the primary tumour is attempted after induction therapy and prior to high-dose myeloablative therapy. Pre-operative imaging is used to define image defined risk factors (IDRF's) for being unable to fully resect the primary tumour (see Table 1.3).

There is conflicting evidence from different groups as to whether the extent of surgical resection impacts on outcome (*Mc Gregor 2005, von Schweinitz 2002, Castel 2002*).

La Quaglia et al found that in 141 high-risk neuroblastoma patients evaluated, local progression was 50% in un-resected compared to 10% in patients with a gross total resection ($p<0.01$). Overall survival was 50% in resected compared to 11% in un-resected patients ($p<0.01$) (*La Quaglia 2004).*

Simon et al looked at the German prospective NB97 trial of patients greater that 18 moths of age with stage 4 disease and correlated the extent of tumour resection with local control rate and outcome. A total of 278 patients were included in the analysis. Following induction chemotherapy, 54.75 of patients underwent a complete surgical resection, 30.6% underwent an incomplete resection and 13.3% had a biopsy only or no surgical resection of the primary tumour. The extent of the surgery had no impact on EFS, LPFS or OS (*Simon 2013).*

Recent unpublished but presented data from the SIOPEN high-risk neuroblastoma trial suggests that extent of surgical resection did have an impact on outcome in this trial (*Holmes 2011*). Overall EFS was 53% at 3 years for those with a complete surgical excision versus EFS of 41% for those with an incomplete resection. With regards to Stage 4 only patients, the 3 year EFS was 46% for completely resected patients versus 38% for those without (p=0.035).

Whether surgical resection is just a surrogate for the biological behaviour of the disease is unknown and this area lacks randomised prospective data.

1.9.2.3 High-Dose Myeloablative Therapy

There were several reported studies in the early 1990's of induction chemotherapy followed by high-dose myeloablative therapy and bone marrow transplantation with mixed results (*Pinkerton 1991,Ohnuma 1995, Garaventa 1996*). Subsequently three randomised control trials were performed as discussed below.

There has been a recently published Cochrane review on high-dose chemotherapy and autologous haematopoietic stem cell rescue for children with high-risk neuroblastoma (*Yalcin* 2010). Three randomised controlled trials comparing myeloablative therapy to conventional therapy in high-risk neuroblastoma were identified (*Matthay 1999, Pritchard 2005, Berthold 2006, Matthay 2009*). This totalled 739 children in these three studies. The meta-analysis of event free-survival performed found a significant difference in favour of the myeloablative treatment approach (HR 0.78; 95% CI 0.67 to 0.90). The meta-analysis of overall survival was also in favour of the myeolablative group (HR 0.74; 95% CI 0.57 to 0.98). The treatment of these patients with myeolablative therapy is likely to be more toxic.

It is not known what the best treatment regime for myeoablative therapy is and it varies between continents. The varying results may be influenced by the other components of multi-modality therapy including the induction regime that is used.

The standard regime in the United States Children Oncology Group studies has been CEM (carboplatin, etoposide and melphalan). Unpublished but presented data from the European SIOPEN high-risk study has shown a significant difference in EFS (3 year EFS 49% vs 35%) and OS (3 year OS 60% v 48%) (p=0.003) in favour of BuMel (Busulfan and melphalan) over CEM in the high-dose myeloablative therapy randomisation. (*Ladenstein et al.* ASCO 2011, ANR Toronto 2012). The severe toxicity rate was higher in the CEM arm. The acute toxicity profile favoured BuMel although the incidence of VOD was higher for BuMel than CEM. The publication of these results is awaited. However, there have been concerns that the EFS for the CEM arm in the European study is less than normally seen in the US group and it has been postulated that CEM may be less effective if a high 'platinum' containing induction regime is used as in the SIOPEN study with Rapid COJEC.

1.9.2.4 External Beam Radiotherapy

Both surgical resection and external beam radiotherapy contribute to obtaining local control in high-risk patients. As discussed above there is some evidence from surgical data that extent of surgical resection and local control can have an impact on outcome for high-risk neuroblastoma patients.

There have been no randomised control trials in Stage M neuroblastoma comparing patients receiving radiotherapy against those with no radiotherapy and most of the radiotherapy data is historical case series. The reported studies have used a variety of doses and techniques with many different high-risk multi-modality protocols used.

Rosen et al. reported on 118 patients with neuroblastoma treated between 1970 and 1980. For the stage IV patients, doses of between 12Gy and 37.5Gy were delivered. In the irradiated group, local failure was reported in 7 out of 22 patients compared to a local failure in 17 out of 21 patients in the non-irradiated group (*Rosen 1984*).

With regards to localised disease, Castleberry *et al.* performed a prospective randomised control trial of chemotherapy +/ - radiotherapy to the primary and regional lymph nodes (24-30Gy in 16-20♯) in patients with Paediatric Oncology Group stage C disease (primary tumour with positive intracavity lymph nodes).

Statistically significant EFS and OS were seen in the combined chemotherapy and radiotherapy arm *(Castleberry 1991).*

The largest reported study of the effect of radiotherapy on local control in high-risk neuroblastoma was by the Children's Cancer Group Study. This was a multi-centre study of 539 patients. The aim of the study was to examine the effect of local radiation given to primary disease sites in high-risk neuroblastoma patients. Patients were treated on protocol CCG-3891 which consisted of chemotherapy, surgery and 10Gy of external beam radiotherapy to gross residual disease and followed by randomisation between continuation chemotherapy (CC) OR autologous bone marrow transplantation. Those receiving ABMT had total body irradiation (3.33Gy x 3#). 5 year loco-regional recurrence rates were 51%+/- 5% for the CC arm and 35%+/-2% for the ABMT patients (p=0.004). For the patients given 10Gy to the primary, the addition of 10Gy of TBI and ABMT resulted in reduced local recurrence (22%+/-12%) compared to the CC arm (52% +/-8%) (p=0.022). Acute toxicity was not increased with EBRT except for increased total parental nutrition requirements. It was therefore postulated that there may be a dose-response relationship for the local EBRT component of therapy (*Haas-kogan 2003*).

A German study examined the value of intensified local radiotherapy (36Gy) to residual tumour volumes in those with residual viable tumour. The initial treatment included induction chemotherapy and high-dose chemotherapy with stem cell transplantation. The 13 patients who received radiotherapy for local residual disease had a similar outcome to those patients without any residual mIBG positive disease.

Those 23 patients without radiotherapy to the residual primary did worse. Multivariate analysis found radiotherapy to be influential on EFS (HR 0.27) and OS (HR 0.17) in addition to MYCN amplification and presence of primary tumour site mIBG residual (*Simon 2006*).

Pai Panandiker *et al.* found that loco-regional tumour control had an impact on overall survival for their cohort of neuroblastoma patients from St. Jude's Children's Research Hospital. Between 2000 and 2006, 44 patients with INSS stage 3 or 4 disease received radiotherapy to the primary tumour at a median dose of 23.4Gy. 11 patients (25%) progressed loco-regionally. The 5 year overall survival was 48.3% for those patients with local control compared to a 5 year OS of 21.8% for those with local failure (p=0.06) (*Pai Panandiker 2010*).

Within the current SIOPEN high-risk neuroblastoma study, external beam radiotherapy is given post-operatively and after high-dose myeloablative therapy to the post-induction chemotherapy tumour volume. It is delivered to the primary tumour bed post-surgery in high-risk protocols with the aim of reducing the risk of local recurrence. Radiotherapy is aimed at any gross residual disease as well as any microscopic residual disease that may be present during surgery.

Traditionally in Europe, radiotherapy has been delivered with a standard parallel-opposed anterior and posterior pair. There is concern that many patients have their radiotherapy dose or target volume coverage reduced to stay within organ tolerance doses and therefore do not receive the protocol dose of radiotherapy. This has been

highlighted by the quality assurance of radiotherapy in the current SIOPEN high-risk neuroblastoma trial (*Gaze 2010, Gaze 2013*).

External beam radiotherapy also has a significant role to play in the palliation of symptomatic metastases and the treatment of spinal cord compression and brain metastases.

External beam radiotherapy is discussed further in chapter 7.

1.9.2.5

Minimal Residual Disease Therapy and Immunotherapy

Patients that have had a good response to induction and consolidation therapy are still at a significant risk of relapse and this is thought to be due to the presence of minimal residual disease (MRD).

High-risk protocols now incorporate a maintenance phase at the end of treatment with the aim of eradicating MRD. This incorporates two different treatment strategies - differentiating agents and immunotherapy.

Differentiating Agents

Retinoids were found to induce differentiation and apoptosis in neuroblastoma cell lines 30 years ago (*Sidell 1982, Reynolds 1991*). Following promise in early phase clinical trials 13-*cis*-retinoic acid was examined in a large prospective phase III trial by the Childrens' Oncology Group. Patients who were post high-dose myeloablative therapy and stem cell transplant were randomised to no further therapy or 6 months of 13-cis-retinoic acid therapy. The 5 year EFS was better for those who received 13-cis-retinoic acid although this did not reach statistical significance. 13-cis-retinoic acid has been incorporated into current high-risk treatment protocols for patients in remission post high-dose therapy (*Matthay 1999, 2009).*

The administration of 13-*cis*-retinoic acid remains problematic in children as it is only available in large capsules and therefore often requires mixing with food for administration. This could have important implications for therapeutic levels of the drug as it vulnerable to degradation by light. The UK Children's Cancer Study Group found a large degree of variability in the pharmacokinetics of 13-*cis*-retinoic acid in children being treated and this could have important implications for toxicity and effectiveness (*Veal 2007*).

There are other retinoids in early phase clinical trials such as Fenretinide, a synthetic retinoid derivative (*Villablanca 2006, Garaventa 2003*). This agent is also only available in capsular form. New Approaches to Neuroblastoma Therapy (NANT) phase I clinical trials examining an intravenous and lipid matrix formulation are underway (*Maurer 2007*).

Immunotherapy

GD2 is a disialoganglioside found on the surface of most neuroblastomas making monocloncal antibodies directed against it an attractive therapeutic option. It has a limited distribution in other tissues – peripheral pain fibres, neurons and melanocytes. There have been several anti-GD2 monoclonal antibodies developed, both murine and humanised. The earliest studies were on the murine antibodies 3F8 and 14G2.

Recent focus has been on studying the human-mouse chimeric antibody, ch.14.18. The activity of this antibody was shown in pre-clinical and early phase trials. The antibody was then combined with IL-2 and GM-CSF in early phase clinical trials. (*Gilman 2009, Ozkaynak 2000*). There were considerable but manageable toxicities in these studies – fever, hypotension, pain and capillary leak.

Ch14.18 has subsequently been studied in combination with GM-CSF and IL-2 in a randomised phase III trial (*Yu 2010*). Patients completing multi-modality therapy for high-risk neuroblastoma were randomised to receive 13-cis-retinoic acid alone or in addition to ch14.18, GM-CSF and IL-2. The trial was stopped early as superior efficacy was seen in the immunotherapy arm. At 2 years there was a statistically significant difference in EFS (66% v 46%, p=0.01) and OS (86% v 75%). There were significant toxicities from the immunotherapy arm, pain grade 3,4 or 5 in 52%, capillary leak syndrome in 25% and hypersensitivity reactions in 25%. Although the outcomes of this trial are the most significant reported for high-risk neuroblastoma, outcomes at 5 years are awaited to see if this significance of EFS and OS persist. Unanswered questions remain however, as we do not know the additional benefit of each of the three treatment components and it is not known whether there is additional benefit of IL-2 to ch14.18 alone. The current European SIOPEN high-risk neuroblastoma trial aims to address this. This is important as the use of immunotherapy, and IL-2 results in significant toxicity for patients.

1.10 Response Assessment

1.10.1 International Neuroblastoma Response Criteria

Universally accepted response criteria are highly desirable as they allow the comparison of clinical trial results across the world and collaborative trial development. Response assessment for neuroblastoma is still based on the International Neuroblastoma Response Criteria as published originally by Brodeur *et al.* in 1988. The original INRC did not take into account mIBG scans but the revised INRC in 1993 did recommend mIBG scanning if it was available although it did not formally appear in the INRC criteria (*Brodeur 1993*).

The original INRC have limitations in accurately defining the response of metastatic sites including bone and bone marrow. The INRC did not define a standard way of assessing response on mIBG scanning. Problems at that time included the universal availability of mIBG scanning, quality of imaging and the fact that not all primaries and metastases are mIBG avid. The INRC gives no guidance on the introduction of new imaging techniques such as ^{18}F-FDG PET or evolving techniques for bone marrow disease quantification.

A revision to the INRC has been proposed and is likely to be introduced within the next couple of years. Julie Park *et al.* presented a consensus statement on a revised INRC at the Advances in Neuroblastoma Research conference in Toronto, June 2012. This revision is likely to involve semi-quantitative scoring of mIBG scans, ^{18}F-FDG PET

for mIBG negative patients, bone marrow assessment by cytology and immunocytology. Urinary catecholamines are likely to be removed from the assessment.

Table 1.5

The International Neuroblastoma Response Criteria, adapted from *Brodeur et al. 1993.*

RESPONSE	PRIMARY TUMOUR	METASTATIC SITES
CR	No tumour	No tumour; catecholamines normal
VGPR	Decreased by 90-99%	No tumour; catecholamines normal; residual ^{99}Tc bone changes allowed
PR	Decreased by >50%	All measurable sites decreased by >50%. Bones and bone marrow: numberof positive bone sites decreased by >50%; no more than 1 positive bone marrow site allowed
MR	No new lesions; >50% reduction of any measurable lesion (primary or metastases) with <50% reduction in any other; <25% increase in any existing lesion	
NR	No new lesions; < 50% reduction but <25% increase in any existing lesion	
PD	Any new lesion; increase of any measurable lesion by >25%; previous negative marrow positive for tumour	

1.10.2 Semi-quantitative scoring of mIBG scans

For the staging and response assessment of mIBG scans, a semi-quantitative scoring method is recommended as it allows quantification of a response and should facilitate comparisons between different organisations and clinical trials internationally. These methods should also improve the concordance between different reporters.

There have been several scoring systems proposed but essentially they all divide the skeleton into anatomical sections and then give each sector a score for extension and intensity.

The two most frequently used are:

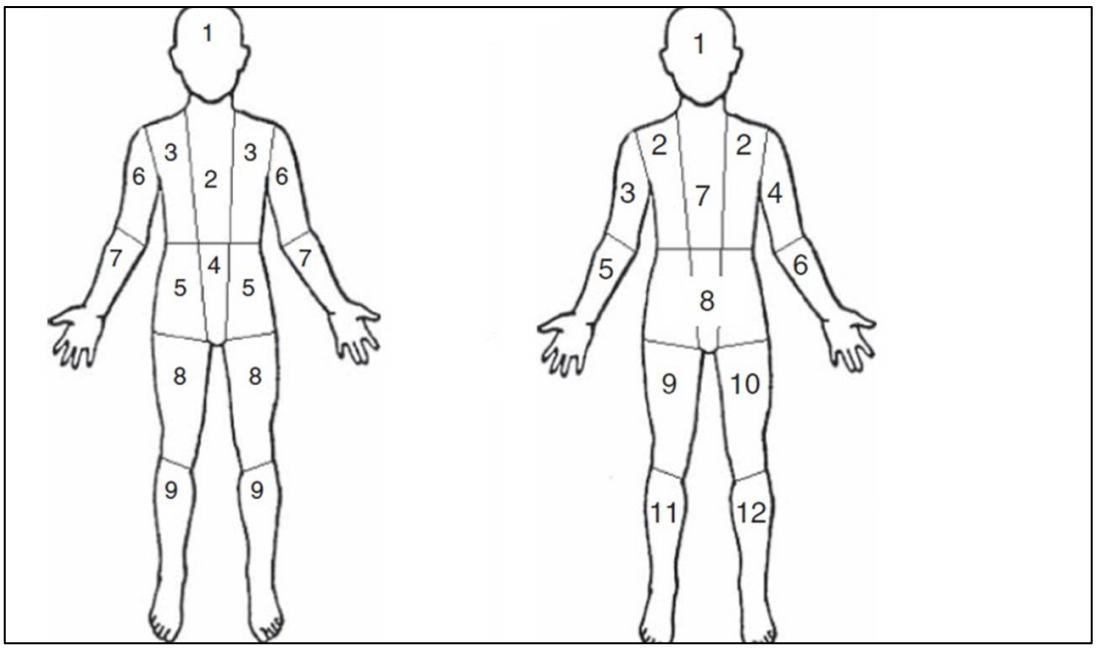
1. The Curie Method – divides the skeleton into 9 segments and adds a 10th segment for any soft tissue involvement. The extension score is given for each segment and graded 0-3: 0 (no sites per segment), 1 (one site per segment), 2 (more than one site per segment), 3 (diffuse involvement, >50% of the segment). Therefore giving a maximum score out of 30. It has been shown to have good concordance between observers. (*Matthay 2003, Messina 2006*). This is the scoring system currently used in North America.

2. The SIOPEN method – divides the skeleton into 12 anatomical segments. The extension score is graded from 0-6 for each segment: 0(no involvement), 1(one discreet lesion), 2 (2 discrete lesions), 3 (3 discreet lesions), 4 (>3 discrete foci or a single diffuse lesion involving <50% of a bone), 5 (diffuse involvement of >50 to 95% whole bone), 6 (diffuse involvement of the entire bone). Giving a maximum score out of 72. This is the method used in Europe. (*Lewington 2009*).

Figure 1.2

The Curie and SIOPEN semi-quantitative mIBG scoring methods

1. The Curie Method 2. The SIOPEN Method



The recent INRG task force report recommends that for response evaluation of mIBG scans the relative extension score by semi-quantitative scoring is calculated by dividing the absolute pre-therapy score by the post –therapy score (*Matthay 2010*). A relative score of 0.5 is considered a partial response; a relative score of 0 is a complete response (*Matthay 2003*).

1.10.3 The prognostic significance of mIBG scores

Recently emerging data has shown that mIBG semi-quantitative scores can have prognostic significance in high-risk neuroblastoma.

Schmidt et al. examined the prognostic significance of ^{123}I-mIBG scans in stage 4 neuroblastoma patients > 1 year of age on the German Neuroblastoma Trial NB97. Patients with residual ^{123}I-mIBG positive disease after four or six cycles of induction chemotherapy had a significantly worse outcome (*Schmidt 2008*).

Other groups have used a semi-quantitative scoring method to evaluate the scans. *Yanik et al*. within the COG study compared semi-quantitative mIBG scores (Curie method) in stage 4 neuroblastoma patients at diagnosis (n=280), post-induction (n=237), post transplant (n=178). They found no correlation between the Curie score at diagnosis and subsequent outcome. Patients with a Curie score >2 after induction therapy had a significantly worse EFS than those with a Curie score ≤2 at the end of induction (3 year EFS 15.4 +/-5.3% vs 44.9 +/- 3.9%, $p<0.001$). Patients with Curie

score >0 post-transplant had a worse 3 year EFS than patients with a score of 0 (28.9+/- 6.8% vs 49.3 +/-4.9%, p=0.009) (*Yanik 2013*).

Despite their differences both the scoring methods were able to predict outcome in the above studies. The Cologne Inter-score comparison study compared the SIOPEN and Curie scoring methods on stage 4 patients treated within the same German NB97 trial. Both scoring systems were found to be equally reliable and predictive (*Decarolis 2013*). Groups form the US and Europe, are currently comparing the scoring systems, and independently evaluating both scoring systems in a large international patient population.

The implications of these findings are that a potential 'ultra-high risk' group of patients could be identified by semi-quantitative scoring of mIBG scans at the end of induction therapy. These patients could then be offered novel therapeutic strategies with the aim of improving their poor outcome.

1.10.4 Detecting Minimal Residual Disease

Increasingly sensitive methods of detecting minimal residual disease (MRD) are being developed using real time RT-PCR markers for example, TH, PHOX2B and DCX. The markers and methods however vary and are being validated in large prospective clinical trials (*Esser 2011,Chambon 2013, Hartomo 2013*). A recent publication from the European HR-NBL1/SIOPEN study evaluated whether the detection of neuroblastoma mRNA's by reverse transcriptase quantitative polymerase chain reaction (RTqPCR) in peripheral blood and bone marrow aspirates at diagnosis or at the end of induction could predict for worse EFS or OS in a cohort of 290 children on the high-risk trial. High levels of TH, PHOX2B or DCX mRNA at diagnosis strongly predicted for a worse EFS and OS. After induction therapy, high levels of mRNA in the bone marrow predicted for a worse EFS and OS but not in peripheral blood (*Viprey 2014*).

1.11 Relapsed and Refractory Disease

Despite intensive multi-modality therapy many high-risk neuroblastoma patients are either primary refractory or relapse further down the line. Less than 40% of high-risk neuroblastoma patients will unfortunately become long-term survivors and therefore the development of more effective treatment strategies for refractory and relapsed patients is essential.

Following relapse, survival is very poor. The Italian neuroblastoma registry has published the outcome of patients within prospective clinical trials, with disease progression or relapse. Overall prognosis was found to be very poor with a median survival of less than 1 year and less than 10% of children surviving for more than 10 years post relapse. For relapsed or progressive disease stage 4 patients the 10 year OS was only 2% (*Garaventa 2009*).

There is no universally accepted approach for patients with relapsed or refractory disease. It is therefore difficult to compare results between clinical trials, as the prior therapy patients will have had will be highly variable.

A recent report from the INRG project examined clinical and biological features predicting survival after relapse. The significant prognostic factors for post relapse survival were age, stage, MYCN status and time from diagnosis to first relapse. Patients relapsing between 6 and 18 months after diagnosis had the lowest overall

survival whereas those relapsing after 36 months or longer had a better overall survival *(London 2011*).

Relapse and refractory strategies involve the use of conventional cytotoxic therapies, new molecular targeted agents or molecular radiotherapy as discussed below.

1.11.1 Conventional Cytotoxic Therapies

Cytotoxic chemotherapy strategies for relapsed or refractory high-risk neuroblastoma have in recent years concentrated on agents such as Irinotecan, Temozolomide and Topotecan. These agents have been examined alone *(Rubie 2006, Vassal 2008*) and subsequently in various combinations with each other, or in combinations with other more established agents (*Garaventa 2003, Simon 2007a, Simon 2007b, Wagner 2009, London 2010, Rubie 2010, Bagatell 2011, Kushner 2011a, Kushner 2011b*).

A recent retrospective analysis of the use of a multi-agent regime of high-dose cyclophosphamide, topotecan and vincristine found major responses (CR/VGPR and PR) statistically more common (52%) in children with a new (first) disease recurrence compared with children with primary refractory disease, or progressive disease that occurred during induction therapy (0%) (*Kushner 2010).*

Repeated courses of cytotoxic chemotherapy are frequently limited by poor bone marrow reserve in this often heavily pre-treated population. Conventional cytotoxic chemotherapies may be reaching their maximum potential in terms of efficacy and toxicity. Therefore, strategies aimed at improving efficacy and reducing toxicity are focusing on new molecular targets.

1.11.2 New Molecular Targeted Agents

Both in adult and paediatric oncology, much research in recent years has focused on developing new molecular agents to target the increasingly diverse set and number of 'drugable' targets that are being identified.

Collaborative research efforts are focusing on rapidly evaluating and screening pre-clinically promising agents that hold potential or have been used with success in adult cancers and bringing them to the paediatric setting for early clinical trials.

Several early phase trials have been conducted including those on Tyrosine kinase inhibitors (*Minturn 2011*), Histone Deacetylase (HDAC) inhibitors (*Fouladi 2010*), Vascular Endothelial Growth Factor Receptor (VEGFR) inhibitors (*Fox 2010a*), β-Tubulin inhibitor *(Fox 2010b*) and mTOR inhibitors (*Spunt 2011, Georeger 2012*). The relationship between different receptor pathways are complex and inhibition at one point often activates another pathway through feedback and so these agents need to be further tested in combination (*Øra 2011).*

Preliminary data suggest that ALK is a therapeutic target for some patients with neuroblastoma. The ALK gene mutation has been found to occur in approximately 2% of patients with a familial predisposition and 8-14% of sporadic neuroblastomas. ALK inhibition with crizotinib has been reported in a phase 1 study (*Mossé 2013*).

There is currently interest in developing randomised Phase II trials using the latest cytotoxics as a 'back-bone' and adding new molecular targets to assess a multimodal molecularly targeted therapy in relapsed high-risk neuroblastoma, for example the BEACON study which is now open in Europe.

The limitations so far of these agents are that blocking one pathway may not be enough and tumours can develop resistance mechanisms to these agents. There is also evidence of inter- and intra tumour heterogeneity in adult oncology making treatment with molecularly targeted agents even more challenging (*Fisher 2013*). This is likely to be the case with neuroblastoma too.

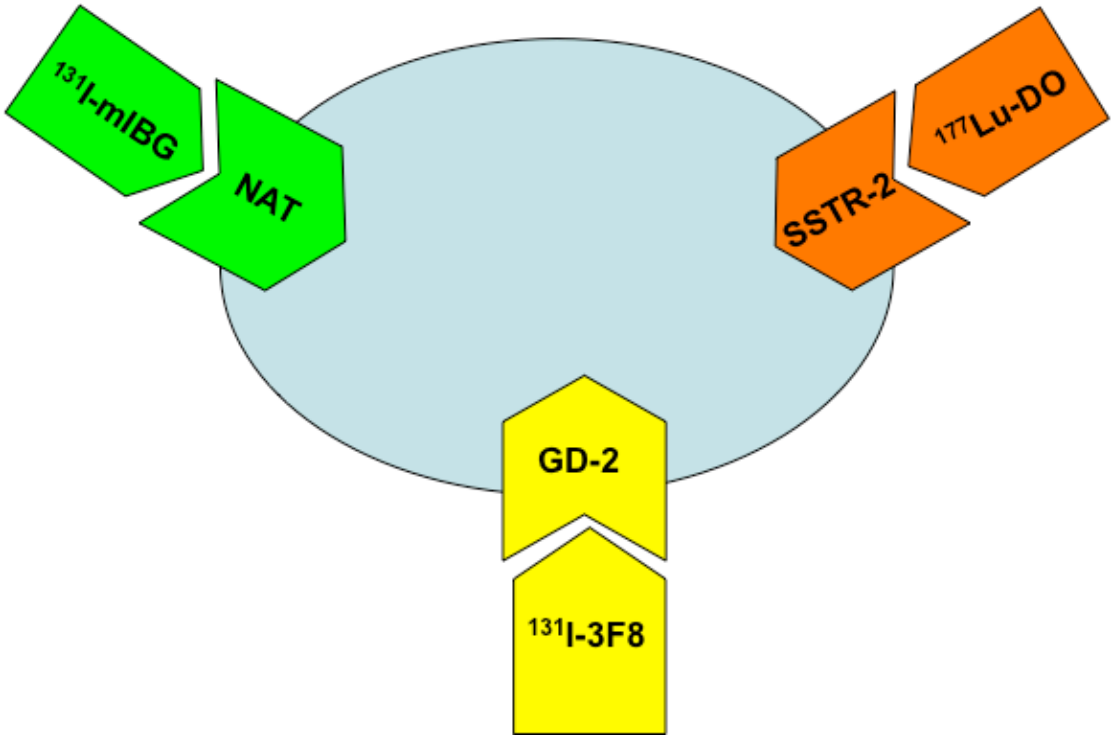
1.11.3 Molecular radiotherapy

Molecular radiotherapy involves giving radiation systemically with the aim of delivering tumoricidal radiation doses to multiple tumour deposits from the same administration. This has important therapeutic implications for a tumour such as neuroblastoma, which is frequently metastatic. Although it is a radiosensitive tumour, and external beam radiotherapy is essential for local control of the primary tumour (as discussed above) it has technical limitations to a tumour that is widely metastatic.

Neuroblastoma expresses a number of distinct and separate biological features, which can act as targets for molecular radiotherapy. These include the disialoganglioside GD2, which can be targeted by a radio-labeled monoclonal antibody, for example 3F8 17 and somatostatin receptor subtype 2, which can be targeted by radiopeptides, for example ^{177}Lutetium DOTATATE (see chapter). The earliest and most frequently used form of molecular radiotherapy in clinical use for neuroblastoma is meta-Iodobenzylguanidine (mIBG) which targets the noradrenaline transporter molecule (NAT).

Figure 1.3

Potential targets for molecular radiotherapy in neuroblastoma.



Meta-iodobenzylguanidine is a guanethidine analogue, which is structurally similar to noradrenaline. ^{131}I-mIBG was first developed at the University of Michigan for the imaging of the adrenal medulla in the late 1970's (*Wieland 1980*). The first imaging studies were performed in dogs and then monkeys (*Wieland 1980, Wieland 1981*). The first imaging of phaeochromocytomas in six human patients with ^{131}I-mIBG was reported in 1981 (*Valk 1981, Sisson 1981*)

^{131}I-mIBG was taken forward for imaging and therapy of neuroblastoma in the mid 1980's. Being a tumour derived from the sympathetic nervous system, neuroblastoma expresses the noradrenaline transporter molecule which takes up

the catecholamine analogue mIBG and approximately 90% of neuroblastomas express NAT.

Most studies using ^{131}I-mIBG have been pilot, phase I or II clinical trials with variable administered activities used and response rates reported. The dose limiting toxicity is myelosupression. Attempts to increase the response to ^{131}I-mIBG have included dose escalation and the addition of radiosensitisers such as topotecan, both of which have used peripheral blood stem cell support to circumnavigate the myelosupression caused by higher administered activities (*Matthay 2006 and 2009, Gaze 2005).* Although most studies in the literature have used ^{131}I-mIBG in the relapsed or refractory setting there are also reports of its use in induction and consolidation stages.

The use of radiolabelled somatostatin analogues for neuroblastoma is presented in chapter 2 and a systematic review of ^{131}I-mIBG therapy in neuroblastoma is presented in chapter 4.

1.12 Special Clinical Scenarios

1.12.1 Spinal Cord Compression

Neuroblastomas may invade the intervertebral foramina and/or the spinal cord in about 5 to 15% of cases. Intra-spinal extension may cause acute neurological symptoms and may also result in long term neurological sequelae affecting quality of life for long-term survivors (*Poretti 2008*).

Patients with spinal cord compression are treated as medical emergencies and urgent commencement of chemotherapy or surgical decompression is required. Retrospective reports have suggested equivalent neurological outcomes for patients treated with primary chemotherapy or surgery with laminectomy but debate remains about the treatment options and treatment algorithms vary. (*Katzenstein 2001, Plantaz, De bernardi 2001, Strother 2006, Yiin 2003, Sandberg 2003*).

Simon et al. have recently published the short and long term outcome of 122 patients with neuroblastoma and spinal cord compression. Patients from four consecutive prospective German clinical trials were retrospectively analysed. They found spinal cord compression to be present in 5% of their studied population. The patients with SCC tended to be younger and have less frequency of MYCN amplification. The authors postulated that this may be because the highly aggressive, often MYCN amplified tumours may have caused other symptoms before

developing SCC. Again no clear advantage was found, for either short- or long- term outcome, for first line treatment with neurosurgery or chemotherapy. Emergency external beam radiotherapy was never used for SCC in this study. The frequency of long-term sequelae in this study was 71% (*Simon 2012*).

1.12.2 Brain Metastases

Many chemotherapy agents do not cross the blood-brain barrier and therefore the central nervous system (CNS) is a potential 'sanctuary site' for neuroblastoma cells. Brain metastases are rare at the time of diagnosis but they do occur at the time of relapse (approximately 10%).

mIBG does not cross the intact blood brain barrier and so diagnostic or surveillance imaging can miss CNS disease. Historically, CNS relapse of neuroblastoma has been universally fatal but earlier recognition of disease in the brain may improve outcome. For diagnosis of brain metastases, an immediate brain and spine MRI is mandatory for patients with symptoms or signs of CNS disease even if the mIBG scan is negative.

There is concern that with the increasing survival rates in high-risk disease that the incidence of brain metastases may increase. A single institutional study looking at patients treated with multi-agent regimes N4, N5, N6 or N7 between 1980 and 1998

found the overall rate of CNS recurrence to be >10%. However, those treated on earlier studies with N4 and N5 had a CNs recurrence incidence of 2% versus 12% for those treated on later regimes with N6 and N7 *(Kramer 2001*).

Historically, the mainstay of treatment for brain metastases has been external beam radiotherapy. Recent developments have examined the use of compartmental radioimmunotherapy using radioiodinated monoclonal antibodies administered intrathecally following surgical resection and external beam radiotherapy. These ^{131}I-labelled monoclonal antibodies are targeted against tumours expressing GD2 or B7H3, which is a glycoprotein preferentially expressed on many tumours compared to normal tissue (*Kramer 2001*).

The team at the Memorial Sloan-kettering Cancer Center has published their retrospective analysis of children with CNS relapse treated with external beam radiotherapy and radioimmunotherapy. Of the 29 children treated, 16 had received craniospinal irradiation (CSI) at a median dose of 21.6Gy and 13 had focal radiotherapy. 14 of the CSI patients also received intra-Ommaya radioimmunotherapy (^{131}I-8H9 or ^{131}I-3F8) but only one patient in the focal radiotherapy group. At a median follow-up of 28 months, 12 patients (75%) in the CSI group were alive without CNS disease. In the focal radiotherapy group all 13 patients had died at a median of 8.8 months (*Croog 2010*). This suggests that the whole neuraxis is at risk in CNS relapse and CSI may be necessary rather than focal irradiation and there may be an additional benefit for intra-ommaya radio-immunotherapy.

The ability to identify those neuroblastoma patients with a high risk of relapse within the CNS would be highly advantageous and allow trials of prophylactic strategies.

1.12.3 Opsoclonus Myoclonus Syndrome

Opsoclonus-myoclonus (OMS) is a chronic and relapsing autoimmune neurological illness, which occurs in 2-3% of patients with neuroblastoma. It is associated with occult neuroblastoma in 40% of cases but there are other causes including infection, metabolic and neoplastic. In neuroblastoma, it is most common in patients under the age of 4 years.

OMS is also known as 'dancing-eye syndrome' and is characterised by multidirectional rapid eye movements (opsoclonus), myoclonus, ataxia and behavioural changes such as sleep problems and irritability.

Children with OMS and neuroblastoma typically have favourable outcomes but the majority will have long-term neurological deficits (*Altman 1976, Pranzatelli 1992, Koh 1994, Russo 1997, Mitchell 2002*).

As the mechanism for developing OMS is thought to be immune mediated most treatments have aimed at being immunosuppressive for example, ACTH,

gammaglobulins, corticosteroids, plasmapharesis, cyclophosphamide and azathioprine (*Petruzzi 1995, Borgna-Pignatti 1996, Veneselli 1998, Yiu 2001*).

More recently treatment with rituximab (a monoclonal antibody that binds to the CD20 antigen on the surface of mature B cells) has been reported in several case reports (*Burke 2008, Alavi 2012, Battaglia 2012*).

1.12.4 Neuroblastoma in teenagers and young adults

Approximately 3–4% of neuroblastoma cases occur in older children, adolescents and young adults (*Conte 2006, Castel 2010*). Neuroblastoma in this age group is generally regarded as having a more indolent course but with a poorer overall survival. The biology and clinical course of neuroblastoma are different in this age group and is reflected in patients' different responses to therapy (*Esiashvili 2007*). As the number of patients in this age group is small, most published reports tend to be small, retrospective and include a heterogeneously treated population.

There have been several recently published cohorts of patients with neuroblastoma within the teenage and young adult population. A group from Milan published 20 years of experience with patients over 12 years old with neuroblastoma. This included 27 patients over 12 years of age treated between 1982 and 2001. Three cases were stage 1, two were stage 2, six were stage 3 and 16 were stage 4. These patients had a long interval between onset of symptoms and diagnosis (mean of 12

months; range 1–24 months) and recurrence and death (median 12 months; range 2–75 months). None of the patients had *MYCN* amplification. Despite a longer course of the disease, this did not translate into a better outcome with an OS for all patients of 40% at 5 years and 20% at 10 years (*Podda 2010*).

In a Spanish cohort of patients with neuroblastoma over 10 years of age, treated between 1992 and 2007, genetic and clinical characteristics were examined. *MYCN* was not amplified in any of the 19 cases studied and no 1p deletions were found. More frequently, 11q deletions and unfavorable histology were observed. The outcome in stage 4 patients (OS of 33%) was worse than for younger children with stage 4 disease. The investigators concluded that the exact age cutoff for older patients has not yet been established and should probably be based on biology (*Castel 2010*).

More recently, the International Neuroblastoma Risk Group Project have published a report on neuroblastoma in older children, adolescents and young adults. They set out to determine if an optimal age cut-off exists that defines indolent disease and if established prognostic factors and treatments used for younger children were applicable to those in the older age group. From their analysis of 4,027 patients over the age of 18 months, older age was found to be prognostic for poorer survival, with outcome worsening gradually with increasing age at diagnosis although there was no specific age cut-found above 18 months. In a multivariable analyses, for patients older than 5 years of age at diagnosis, factors prognostic for lower EFS and OS were INSS stage 4, MYCN amplification and unfavourable INPC histology. For older

patients with stage 4 neuroblastoma there was a significant EFS and OS in those that had received an autologous hematopoietic cell transplant (*Mossé 2014*).

With regards to adult patients with neuroblastoma, a Sorrentino et al have recently published the Italian experience of 21 adult patients. EFS at 10 years was worse for all stages compared to younger patients – stage 1/2 33.3%, stage 3 16.7%, stage 4 0%. All 6 stage 4 patients died form disease and the overall survival for all patients was 39.8% (*Sorrentino 2014*).

As well as a different biology of disease in the adolescent and young adult population there is also evidence that this age group responds differently to therapy, with more chemoresistant disease. A retrospective analysis of a treatment regimen of high-dose cyclophosphamide, topotecan and vincristine found statistically significant reduced activity in adolescents and adults compared with children (*Kushner 2010*).

Because of a lack of separate treatment recommendations for this age group with neuroblastoma they are often treated according to pediatric guidelines. It is essential, therefore, to examine alternative treatment strategies, such as ^{131}I-mIBG. In a large Phase II trial of ^{131}I-mIBG, univariate analysis indicated that age >12 years at the time of treatment was a positive prognostic factor for response, compared with patients who were aged 22 months to 6 years (*Matthay 2007*). More recently, a published retrospective analysis on ^{131}I-mIBG in adolescents and young adults found an overall response rate of 46%. Patients over 18 years of age at first ^{131}I-mIBG

therapy had a higher response rate (56%) than those between 10 and 17 years of age (39%) (*Polishchuk 2011*). Therefore, even within the teenage and young adult population, there is variation in response to therapy depending on age.

The concept of this type of indolent or smoldering disease should however not be limited just to the teenage and young adult population as there is also a cohort of children who develop this kind of disease. Kushner et al published their experience of a cohort of children with active neuroblastoma > 5 years from diagnosis (*Kushner 2002*). This chronic type of disease is rare in children with MYCN amplification. The effect of improved initial therapies and more options for relapsed disease in the last decade is likely to have had an impact.

It is essential that new therapeutic approaches are developed for the teenage and young adult age group and their development is likely to come with increased understanding of the biology of the disease. The need for specific clinical guidelines for adolescents and adults with neuroblastoma is gaining international recognition.

1.14 Late effects of treatment

As discussed previously the prognosis for high-risk neuroblastoma is poor and therefore there is a paucity of data on late effects of treatment especially with regards to the high-intensity strategies currently being used. Survivors of high-risk neuroblastoma, are likely to face the huge consequences of intensive multi-modality therapy often given at an early age.

A retrospective report from the Memorial Sloan-Kettering Cancer Center, published in 2005, looked for late effects in patients previously treated for advanced stage neuroblastoma (stage 3 or 4). The study included 63 survivors with a mean age of 3 years at diagnosis and a median follow up from diagnosis of 7.06 years. All of the patients had received surgery and chemotherapy, 89% external bema radiotherapy, 62% immunotherapy and 56% autologous stem cell transplantation. Late complications were detected in 95% of the survivors in their cohort. The most frequent complications were hearing loss (62%), primary hypothyroidism (24%), ovarian failure (41% of females), musculoskeletal (19%) and pulmonary (19%) *(Laverdière 2005).*

A recent publication by Cohen et al reports on a retrospective evaluation of 51 patients, treated between 1994 and 2007, with high-risk neuroblastoma who had survived for greater than 1 year following intensive multimodality treatment including aggressive chemotherapy, surgery, local external bema radiotherapy and

single or tandem autologous stem cell transplantation. Of note the majority of patients received tandem transplantation with chemotherapy conditioning for the first and melphalan and 12Gy Total Body Irradiation for the second. There was a median follow up of 6.1 years. Patient's linear growth was significantly impacted with mean change in height z-scores from pre-treatment to last clinic visit of -1.91 in those that had received TBI and-0.77 in those who had not (p=0.003). Pre-diabetes or diabetes was seen in 50%, hypothyroidism in 59% and ovarian insufficiency in 75%. Hearing loss was seen in 73% and cardiac dysfunction in 23%. 14% had scoliosis and dental issues were common (51%). 29% of patients required special educational needs and 4% developed a second malignancy (*Cohen 2014*).

1.15 The Radiobiology of Neuroblastoma

Human tumours show wide variation in their response to radiation with some tumours such as seminoma being highly radiosensitive and others such as melanoma being relatively radioresistant. There is both laboratory and clinical evidence to suggest that neuroblastoma is a relatively radiosensitive disease.

Deacon et al. examined the radiation response of a neuroblastoma xenograpft HX138 in vitro and in vivo using single cells in suspension, multicellular spheroids and xenografts in immune suppressed mice. The results following irradiation of cells from all three different experimental systems were consistent with it being a highly radiosensitive tumour. The results from split-dose experiments showed some evidence or repair of sub-lethal damage. Tumour cells irradiated as spheroids or in vivo were more resistant than single cells. Radio-resistance was seen when tumours were irradiated under conditions of hypoxia. The lack of a shoulder on the single-dose survival curve and a limited capacity of repair in split-dose experiments led the authors to postulate that the use of multiple small fractions or low dose-rate irradiation may be used to exploit the greater repair capacity of normal tissues (*Deacon 1985*). Clinically, *Jacobsen et al.* in 1983 studied children with Evans stage II or III disease who received post operative radiation 900 to 4500 rad and found no patient had a local recurrence or died of disease. 9 of 15 patients received doses of 900-1500 rad only.

1.16 Scope of this study

Neuroblastoma is a hugely diverse disease but the outcome for high-risk patients is still universally very poor and the long-term survival rate remains under 40%. It is a relatively radiosensitive tumour and radiotherapy has a significant role to play in the management of high-risk disease - both external beam and molecular radiotherapy.

The aim of this study is to examine potential ways of improving the outcomes for high-risk neuroblastoma through the optimisation of these radiotherapeutic techniques. These strategies are summarised below and described in detail in chapter 2-7.

Radiolabelled somatostatin analogues have been successfully used in the treatment of adult neuroendocrine tumours and somatostatin receptors and have been shown to be expressed by many neuroblastomas. Targeting somatostatin receptors expressed by many neuroblastomas with radiolabelled somtostatin analogues gives a new, distinct and separate target to that used with mIBG. This can be exploited for both imaging (with ^{68}Gallium DOTATATE) and therapy (with ^{177}Lutetium DOTATATE). The use of ^{68}Ga-DOTATATE PET/CT and ^{177}Lutetium DOTATATE, will be reported here for the first time in relapsed and refractory neuroblastoma and the planned phase II LuDO trial presented (see Chapter 2).

mIBG and radiolabelled somatostatin analogues target different molecular receptors expressed on the surface of neuroblastomas. The frequency of expression of the NAT and sstr2 and their heterogeneity of distribution within a cohort of neuroblastoma tissues will be examined by immunohistochemistry (see Chapter 3).

Molecular radiotherapy in the form of ^{131}I-mIBG has been used in the treatment of relapsed and refractory neuroblastoma for over 25 years but a randomised control trial incorporating it has never been performed. A systematic review of ^{131}I-mIBG therapy in neuroblastoma is therefore presented. The aim of the systematic review is to increasing the understanding of the role of ^{131}I-mIBG and to highlight questions that should be addressed in future, prospective and randomised clinical trials (see Chapter 4).

For children undergoing molecular radiotherapy it is necessary for them to have a parent, relative or other responsible adult who can provide comfort and care for them during their treatment. These so called 'comforters and carers' must consent to provide the care and they will inevitably receive a dose of radiation from providing that care. Doses received by comforters and carers whilst looking after children receiving molecular radiotherapy with ^{131}I-mIBG, ^{131}I-NaI and ^{177}Lu-DOTATATE will be presented (see chapter 5).

Although ^{123}I-mIBG imaging is the gold standard imaging modality in neuroblastoma the role of ^{18}F-FDG PET/CT in neuroblastoma remains undefined. A comparison of

^{123}I-mIBG scans and ^{18}F-FDG PET/CT scans in a cohort of patients receiving ^{131}I-mIBG molecular radiotherapy for neuroblastoma is reported (Chapter 6).

Within high-risk neuroblastoma multi-modality treatment protocols, external beam radiotherapy to the primary tumour bed is given with the aim of improving local control. This has historically been delivered using conventional radiotherapy fields but often a compromise has to be made on tumour volume coverage with these techniques. Over the last decade there have been significant technical advances made in external beam radiotherapy including Intensity Modulated Radiotherapy (IMRT) and its derivatives such as Intensity Modulated Arc Therapy (IMAT). A planning study comparing conventional radiotherapy and an IMAT technique in abdominal neuroblastoma is reported examining whether IMAT offers advantages for tumour volume coverage (chapter 7).

Chapter 2

Radiolabelled somatostatin analogues for the imaging and treatment of neuroblastoma

2.1 Introduction

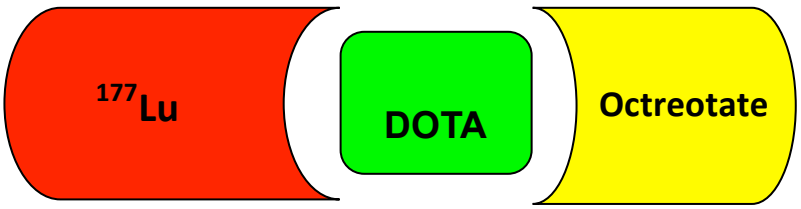
The outcome for patients with relapsed or refractory neuroblastoma remains very poor and new therapeutic treatment options are therefore required. Cytotoxic chemotherapy has been used in the relapsed and refractory disease setting but response rates have been suboptimal due to drug resistance and toxicity, mainly bone marrow suppression (see chapter 1).

Neuroblastoma is a radiosensitive disease and molecular radiotherapy can deliver tumour specific radiation targeting multiple sites of disease from the same administration, important in metastatic neuroblastoma. Molecular radiotherapy has played an important role in the management of neuroblastoma since the mid 1980's in the form of mIBG. mIBG is a guanethidine derivative that targets the noradrenaline transporter molecule (NAT) expressed by many neuroblastoma cells. It can be labelled with ^{123}I for imaging and ^{131}I for therapy. Its main toxicity is myelosupression and attempts to increase response rates have included the use of higher administered activities, the use of radiosensitisers or both with peripheral blood stem cell support to circumvent the myelosupression. Although there is some evidence of good response rates to ^{131}I-mIBG, its ultimate role and effectiveness remain undefined (see chapter 4).

Radiolabelled somatostatin analogues can be used as radiopharmaceuticals to target somatostatin receptors for diagnostic imaging and for peptide receptor radionuclide therapy (PRRT). Radiolabelled somatostatin analogues have an established role in the imaging, staging and response assessment as well as for therapy in metastatic, somatostatin positive neuroendocrine tumours in adults.

All radiolabelled somatostatin analogues have a radionuclide (eg. ^{177}Lutetium, ^{90}Yttrium, ^{68}Gallium), a somatostatin analogue (eg. Octreotate or Octreotide) and a chelating agent (eg. DOTA) (see Figure 2.1).

Figure 2.1 Illustration of the components of ^{177}Lu-DOTATATE.



Octreotate, the somatostatin analogue, has a high affinity for somatostatin receptor type-2 and can be labelled with the PET agent, ^{68}Gallium, for imaging or labelled with ^{177}Lutetium for therapy.

Before PRRT therapy was utilised in adult neuroendocrine tumours, imaging of somatostatin receptor positive tumours with [^{111}In-DTPA^{0}] octreotide (Octreoscan) was established. Attempts at treating metastatic neuroendocrine tumours with high dose [^{111}In-DTPA^{0}] octreotide were then made. Initial studies were encouraging but partial remissions were not seen (*Valkema 2002*). This was due to [^{111}In-DTPA^{0}] octreotide, in addition to the gamma component used for imaging, primarily emitting Auger electrons which have a very short range (1-10 nm), and therefore requiring intracellular localisation to cause double strand breaks in DNA.

The next development was a modified somatostatin analogue [Tyr3]octreotide with a higher affinity for the SSTR-2 and a different chelator, DOTA, which enabled a more stable binding to the radionuclide ^{90}Yttrium. There have been several Phase I and II trials in adults with somatostatin positive neuroendocrine tumours using [^{90}Y-DOTA^{0}, Tyr3]octreotide with Complete Responses and Partial Responses in 10-30% of patients although there have been many differences in the protocols and administered activities used with minimal dosimetry performed (*Otte 1999, Chinol 2002, Paganelli 2002, Valkema 2003*).

The somatostatin analogue [DTPA^{0}, Tyr^{3}]octreotate differs form [DTPA^{0}Tyr^{3}]octreotide in that the C-terminal threoninol is replaced with threonine. It has been shown to have a greater binding to somatostatin positive tumours in animal experiments (*De Jong 1998).* Its DOTA-coupled counterpart [DOTA^{0}Tyr^{3}]octreotate labelled with ^{177}Lu was initially very effective in tumour regression in a rat model (*Erion 1999*). There was a reported nine fold increase in affinity for the somatostatin receptor subtype 2 for [DOTA^{0},Tyr^{3}]octreotate when compared with [DOTA^{0},Tyr^{3}]octreotide in vitro (*Reubi 2000*).

Kwekkeboom *et al.* compared the uptake of radioactivity of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and [^{111}In-DTPA^{0}]octreotide and found comparable uptake in liver, spleen and kidneys but 3-4 fold higher uptake in the tumours in the [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate patients thereby, increasing the therapeutic window (*Kwekkeboom 2001*).

There is a lower tissue penetration range for ^{177}Lu as compared to ^{90}Y which may be important for larger tumours. There have been no randomized trials comparing [^{90}Y-DOTA^{0},Tyr^{3}]octreotide and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

The largest published study of ^{177}Lutetium DOTATATE in adults with metastatic gastroenteropancreatic neuroendocrine tumours is by Kwekkeboom *et al* (*Kwekkeboom 2008*). They reported toxicity analysis in 504 patients and efficacy analysis in 310 patients. Patients were selected for therapy if the tumour uptake was at least as high as the uptake in the liver on [^{111}In- DTPA0]octreotide scintigraphy (OctreoScan). Patients received ^{177}Lutetium DOTATAE at a fixed administered activity of 7.4GBq with up to 4 administrations being given with a 6-10 week interval. Although there is no specific dosimetry recorded in the publication, it states that patients were treated up to a cumulative dose of 750 to 800 mCi (27.8 to 29.6 GBq; corresponding with a radiation dose to the bone marrow of 2 Gy), unless dosimetric calculations indicated that the radiation dose to the kidneys would then exceed 23 Gy; in these cases the cumulative dose was reduced to 500 to 700 mCi. Any haematological toxicity grade 3 or 4 occurred in 3.6% of administrations. Complete response was seen in 2%, partial response in 28% and minor response in 16% of patients. There was a survival benefit of 40 to 72 months from diagnosis compared to historical controls.

Bodei *et al.* have also published the results of a phase I-II trial of ^{177}Lutetium DOTATATE in SSTR2 expressing neuroendocrine tumours in 51 patients. No major acute or delayed renal or haematological toxicity was seen (one grade 3 leukopenia and thrombocytopenia). Partial and complete responses were seen in 32.6%. Overall survival was 68% at 36 months (*Bodei 2011*).

In these adult studies, ^{177}Lutetium DOTATATE has been well tolerated with a low incidence of haematological and renal toxicity (the main side effects reported).

Renal irradiation occurs by the proximal tubular reabsorption of the radiolabelled somatostatin analogue and co-administration of amino acid solution has been shown to reduce the risk of renal damage, as has limiting the estimated cumulative dose to the kidneys to 23Gy or less (*Bernard 1997, Bodei 2003*).

As can be seen in Table 2.1, ^{90}Y has a long particle range and a higher β mean energy and therefore is most suitable in theory for large metastatic deposits. In contrast, ^{177}Lu has a much lower mean β energy and particle range making it more suitable for the treatment of small metastases. For these reasons it has been hypothesised that a combination of both therapies may be superior to either used alone. This has been examined clinically in the adult neuroendocrine tumour population. *Villard et al.* published their results of a cohort study of a total of 486 patients with metastatic neuroendocrine tumours to compare the effectiveness of single versus combination therapy. 237 patients received [^{90}Y-DOTA]-TOC and 249 patients received [^{90}Y-DOTA]-TOC + [^{177}Lu-DOTA]-TOC. Patients receiving the

combination therapy had an improved overall survival compared to the single therapy patients (*Villard 2012*).

Table 2.1 Physical properties of the three most commonly used β emitting radionuclides used for molecular radiotherapy

	^{131}I	^{90}Y	^{177}Lu
Half Life	8.04 days	2.67 days	6.7 days
Emissions	β and γ	β	β and γ
Mean β energy (MeV)	0.2	0.94	0.15
Mean particle range (mm)	0.45	4.2	0.27

^{90}Y is a pure beta emitter and therefore historically dosimetry and imaging have required concurrent administration of ^{111}In-DTPA-D-Phe^{1}-octreotide whereas ^{177}Lu-DOTATATE emits gamma and beta radiation, allowing essential dosimetry measurements and imaging to be performed from the same therapeutic administration.

To select suitable patients for molecular radiotherapy with radiolabelled somatostatoin analogues, ^{68}Gallium DOTATATE PET/CT has become the gold standard imaging modality in adult patients with somatostatin positive neuroendocrine tumours. It has been shown to have a higher sensitivity and improved spatial resolution for the detection of somatostatin receptor positive tumours compared to somatostatin receptor scintigraphy with conventional single photon emission computed tomography (SPECT) gamma camera

imaging using for example, [^{111}In-DTPA^{0}]octreotide (Octreoscan®) (*Kowalski 2003, Buchman 2007, Gabriel 2007, Kayani 2008*). ^{68}Ga-DOTATATE also has a high affinity for somatostatin receptor subtype-2, enabling selection of patients for therapy with radiolabelled somatostatin analogues (*Reubi 2000, Reubi 2003*).

^{123}I-mIBG remains the gold standard imaging modality for the diagnosis, staging and response assessment of metastatic neuroblastoma. However, it does have limitations, including poor spatial resolution and obvious difficulties in the 10% of neuroblastoma patients with mIBG negative disease.

Many neuroblastomas also express somatostatin receptors. There are five subtypes of somatostatin receptor, SSTR1-5, but the most frequently expressed in neuroblastoma tissues has been somatostatin receptor type-2 (SSTR2) (*O'Dorisio 1994, Georgantzi 2010*).

The SSTR-2 gives a separate and distinct, alternative molecular target to the Noradrenaline Transporter molecule (NAT) that is targeted by mIBG. The frequency of SSTR2 expression has been reported to be between 80-90% of neuroblastomas studied (*Albers 2000, Georgantzi 2010*) but we have found it to be highly variable in our own series (see chapter 3).

The work in this chapter reports on the radiolabelled somatostatin analogue, ^{68}Ga-DOTATATE PET/CT, for imaging of neuroblastoma as well as the first pilot study on the use of ^{177}Lutetium DOTATATE for neuroblastoma and the development of a phase II trial to look at efficacy and toxicity of ^{177}Lutetium DOTATATE in refractory and relapsed neuroblastoma.

2.2 A comparison of ^{68}Ga-DOTATATE and ^{123}I-mIBG for disease assessment in high-risk neuroblastoma

2.2.1 Materials and Methods

High-risk neuroblastoma patients who had been imaged with both ^{68}Ga-DOTATATE PET/CT and ^{123}I-mIBG were identified. Patients were imaged at diagnosis or when they had primary refractory or relapsed disease. Patients with three month or greater gap between the two scans were excluded. If a patient had more than one set of paired scans (e.g. before and after treatment) only one set (typically that before treatment when the disease burden was greater) was used.

For the ^{68}Ga-DOTATATE PET/CT, all patients were imaged according to local protocol (*Virgolini 2010)* on a Discovery STE PET/CT system (GE Healthcare). Patients were injected intravenously with at least 100MBq for adequate image quality. Imaging took place at least 45 minutes but no longer than an hour after injection. A low-dose scout projection (120kVp; 10mA; pitch, 1.75) was used to localize the region of head to thigh for transmission and emission imaging. CT was performed at 140kVp and 40mA for paediatrics. PET was performed in 3-dimensional mode with 4 min per bed position. Iterative reconstruction with 21 subsets was performed with attenuation correction.

Prior to ^{123}I-mIBG imaging, all patients received thyroid blockade by oral administration of Potassium Iodide. In addition, all other medicines known to interfere with tumour uptake of radiolabelled mIBG were discontinued. UCLH patients were imaged on a GE Infinia Hawkeye 4 gamma camera system. Imaging was performed at 4 and 24 hours post-injection. Whole-

body images were obtained at both time-points and SPECT/CT images acquired immediately after the 24 hour whole body image. Whole-body images were obtained using low-energy high-resolution collimators with a 20% energy window centred over a 159keV photopeak. SPECT/CT images were acquired with an additional scatter window centred over 130keV. Projection time was 40s and 120 projections acquired, with the region to be evaluated assessed from the patient history and whole-body image. Localisation was aided with a planar x-ray scout view. Following SPECT, low-dose CT was acquired with slice thickness of 1mm. Data was reconstructed using iterative reconstruction (20 iterations) with attenuation correction derived from CT. Scatter correction was also applied. There were patients who had for logistical reasons ^{123}I-mIBG imaging in other nuclear medicine departments.

The uptake pattern of ^{68}Ga-DOTATATE PET/CT with maximum-intensity projection (MIP) with ^{123}I-mIBG whole-body scan (24 hour scan) were then compared. The scans were evaluated by two semi-quantitative scoring systems to fully map the extent of disease in bone/bone marrow and soft-tissue.

To assess the disease extent in the skeleton we used the SIOPEN semi-quantitative scoring system (*Lewington 2009*). The score divides the skeleton into 12 anatomical segments. The extension score is graded from 0-6 for each segment: 0 (no involvement), 1 (one discreet lesion), 2 (2 discrete lesions), 3 (3 discreet lesions), 4 (>3 discrete foci or a single diffuse lesion involving <50% of a bone), 5 (diffuse involvement of >50 to 95% whole bone), 6 (diffuse involvement of the entire bone). This therefore gives a maximum score out of 72.

The SIOPEN scoring system was devised for scoring mIBG scans and does not include soft tissue disease. There are other semi-quantitative scoring systems in use such as the Curie system (see Chapter 1). The SIOPEN system was chosen in this situation, as we are most familiar with it, it has more anatomical segments than the Curie system and a wider range of scores so potentially allowing a greater degree of discrimination.

For the soft tissue score, a soft tissue scoring system for the primary tumour and other soft tissue locations was used giving a total score total out of 5: uptake in primary (0-1), uptake in liver/lung (0-2), uptake in other soft tissue site (0-2). This is a similar approach as published by Papathanasiou *et al.* (2011). There is no recognised semi-quantitative scoring system for ^{68}Ga-DOTATATE PET/CT's so the same SIOPEN scoring system was used for the comparison of the two imaging modalities. The SIOPEN semi-quantitative scoring system only utilises data form planar scintigraphy. To enable a fair comparison between the scans we therefore used the MIP from the ^{68}Ga-DOTATATE scans. The scans were also given a comment on overall concordance of the lesions between the two scans.

An overall assessment score was given as follows:

1 –^{123}I-mIBG better than ^{68}Ga-DOTATATE at mapping disease extent

2 – ^{68}Ga-DOTATATE better than ^{123}I-mIBG at mapping disease extent

3 – ^{68}Ga-DOTATATE and ^{123}I-mIBG equal at mapping disease extent

Scoring of the scans was done jointly by a nuclear medicine physician and myself, and checked by a second nuclear medicine physician, with any disagreement resolved by consensus.

2.2.2 Results

There were 32 high-risk neuroblastoma patients eligible for the study and their characteristics are shown in table 2.2.

Table 2.2 Characteristics of patients with paired ^{123}I-mIBG and ^{68}Ga-DOTATATE scans

Patient No.	Age	Sex	Disease Status	MYCN
1	7	M	Stage M – relapse	N
2	5	M	Stage M – relapse	N
3	27	F	Stage M – relapse	N
4	24	F	Stage M – relapse	N
5	18	M	Stage M (LN only) - refractory	N
6	15	M	Stage M - refractory	N
7	14	F	Stage L2 - diagnosis	Y
8	9	F	Stage M – relapse	N
9	7	F	Stage M – relapse	N
10	9	M	Stage M –refractory	N
11	7	M	Stage L2 – relapse	N
12	7	M	Stage M – refractory	N
13	8	F	Stage M – relapse	Y
14	5	M	Stage M – relapse	N
15	6	F	Stage M – refractory	N
16	3	M	Stage M – refractory	N
17	6	M	Stage M – refractory	N
18	3	M	Stage M – relapse	Y
19	46	M	Stage M – relapse	N
20	14	M	Stage M – diagnosis	N
21	4	F	Stage M – relapse	N
22	5	F	Stage M – relapse	N
23	7	F	Stage M – relapse	N
24	9	F	Stage M- relapse	N
25	8	M	Stage M - relapse	N
26	15	F	Stage M - relapse	N
27	15	F	Stage M - relapse	N
28	9	F	Stage M- relapse	N
29	21	M	Stage M - relapse	N
30	4	M	Stage M - relapse	N
31	10	F	Stage M - refractory	N
32	2	M	Stage M - refractory	N

There were 17 male and 15 female patients with a median age of 8 years (range 2-46 years).

Table 2.3 The SIOPEN and soft tissue scores for ^{123}I-mIBG and ^{68}Ga-DOTATATE scans

	^{123}I-mIBG scores		^{68}Ga-DOTATATE scores		Concordant/ Discordant	Overall Assessment Score
Patient No.	SIOPEN	Soft Tissue	SIOPEN	Soft Tissue		
1	8	1	35	2	D	2
2	23	0	52	0	D	2
3	8	2	17	2	C	2
4	0	1	2	1	D	2
5	0	2	0	2	C	3
6	4	3	26	4	D	2
7	0	1	0	2	C	3
8	50	0	51	0	C	3
9	6	1	0	1	D	1
10	0	0	2	1	D	2
11	0	0	0	1	D	2
12	0	1	0	1	C	3
13	0	1	0	1	C	3
14	6	1	19	1	D	2
15	0	2	6	1	D	2
16	2	2	2	3	D	3
17	9	1	20	1	D	2
18	0	1	0	1	C	3
19	8	0	12	0	D	2
20	24	1	13	1	C	1
21	1	1	2	1	C	3
22	1	0	1	0	C	3
23	14	1	39	1	D	2
24	4	0	43	0	D	2
25	22	1	21	1	D	3
26	28	0	36	0	D	2
27	0	1	5	2	D	2
28	24	0	30	1	D	2
29	36	0	43	0	C	2
30	1	0	13	1	D	2
31	11	0	12	0	D	3
32	53	1	53	1	C	3

D = discordant; C= concordant

^{68}Ga-DOTATATE PET/CT was positive in all 32 patients. The ^{123}I-mIBG scan was positive in 30 patients and negative in 2 patients. Overall the two imaging modalities were discordant in 19/32 patients.

^{68}Ga-DOTATATE PET identified bone lesions in 25/32 patients compared to 22/32 for ^{123}I-mIBG. The mean ± SD SIOPEN scores were significantly lower for the ^{123}I-mIBG than the ^{68}Ga-DOTATATE (10.7 ± 14.5 v's 17.3 ± 17.9; p<0.002) and a significant positive correlation between the two scores was found (Pearson's r=0.794, <0.001).

Figure 2.2 Correlation between the SIOPEN scores on ^{68}Ga-DOTATATE PET and ^{123}I-mIBG.

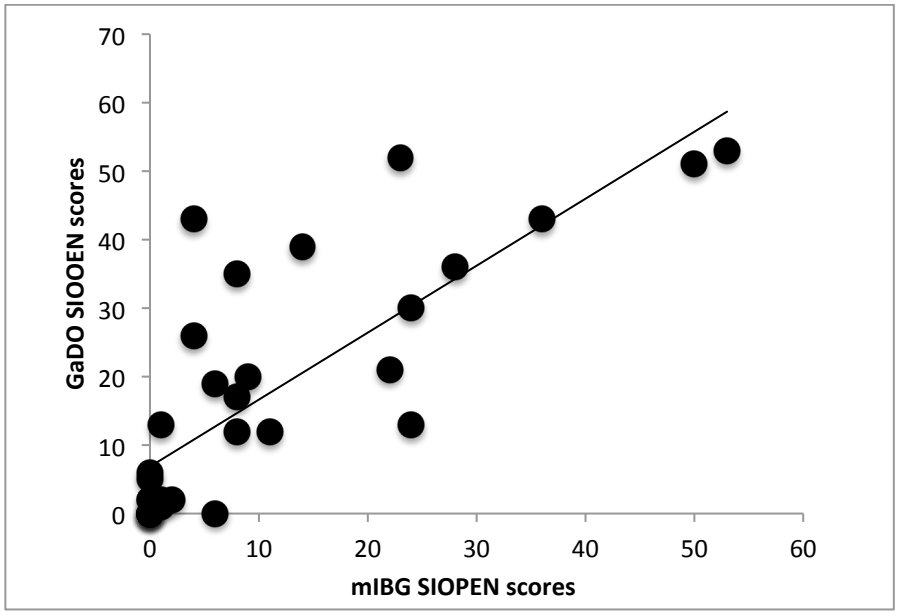
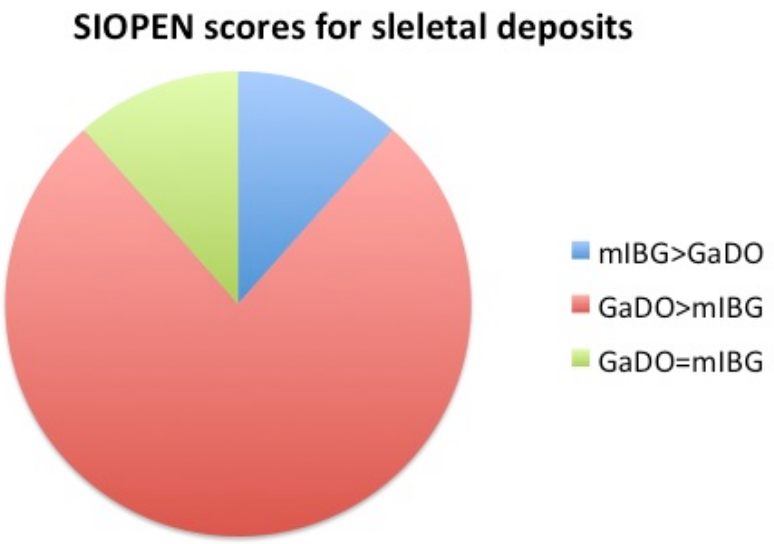


Figure 2.3

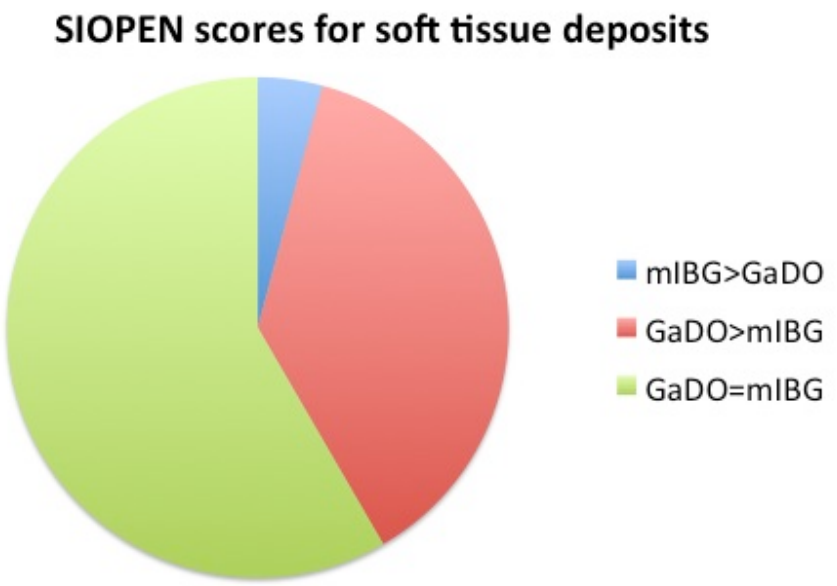
The SIOPEN skeletal scores were: ^{68}Ga-DOTATATE = ^{123}I-mIBG in 3/26, ^{68}Ga-DOTATATE <^{123}I-mIBG in 3/26 and ^{68}Ga-DOTATATE >^{123}I-mIBG in 20/26 patients.



^{68}Ga-DOTATATE identified soft tissue lesions in 24/32 patients compared to ^{123}I-mIBG in 20/32 patients. No significant difference was found between the soft tissue scores (mean ± SD ^{123}I-mIBG 0.81 ± 0.78 v's ^{68}Ga-DOTATATE 1.06 ± 0.91; Wilcoxon rank test p=0.06). A significant positive correlation was found between the two soft tissue scores (Spearman r=0.795, p<0.001).

Figure 2.4

The soft tissue scores were: ^{68}Ga-DOTATATE=^{123}I-mIBG in 14/24, ^{68}Ga-DOTATATE<^{123}I-mIBG in only 1/24 and ^{68}Ga-DOTATATE >^{123}I-mIBG in 9/24 patients.



Figures 2.5 – 2.13 Clinical examples of the different uptake patterns seen for patients on the ^{123}I-mIBG scan and ^{68}Ga-DOTATATE PET.

Figure 2.5

Patient 32: equal SIOPEN scores for skeletal deposits (score=53) and soft tissue scores (score=1) on ^{123}I-MIBG (A) and ^{68}Ga-DOTATATE PET (B).

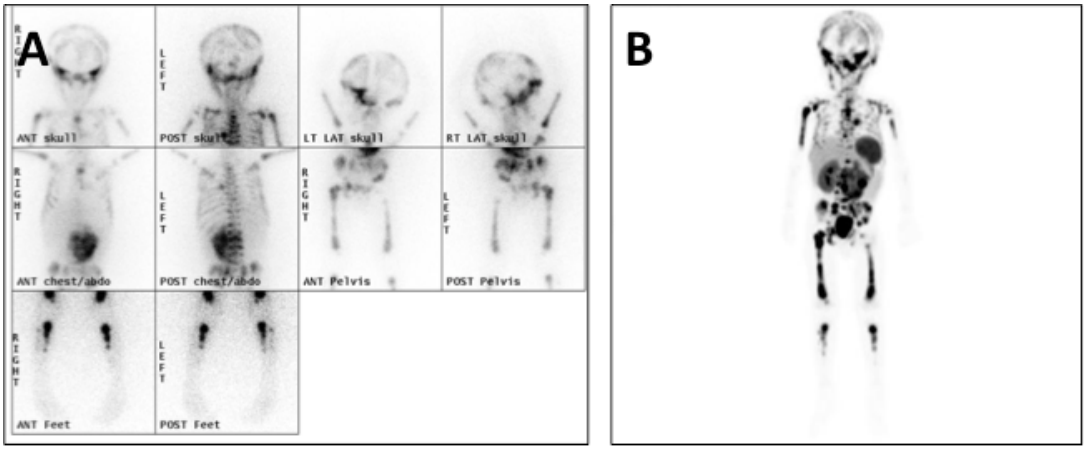


Figure 2.6 Patient 8: similar SIOPEN skeletal scores on ^{123}I-mIBG (score=50) (A) and ^{68}Ga-DOTATATE PET (score=51)(B) and the areas of uptake were concordant. The soft tissue scores were 0 on both imaging modalities.

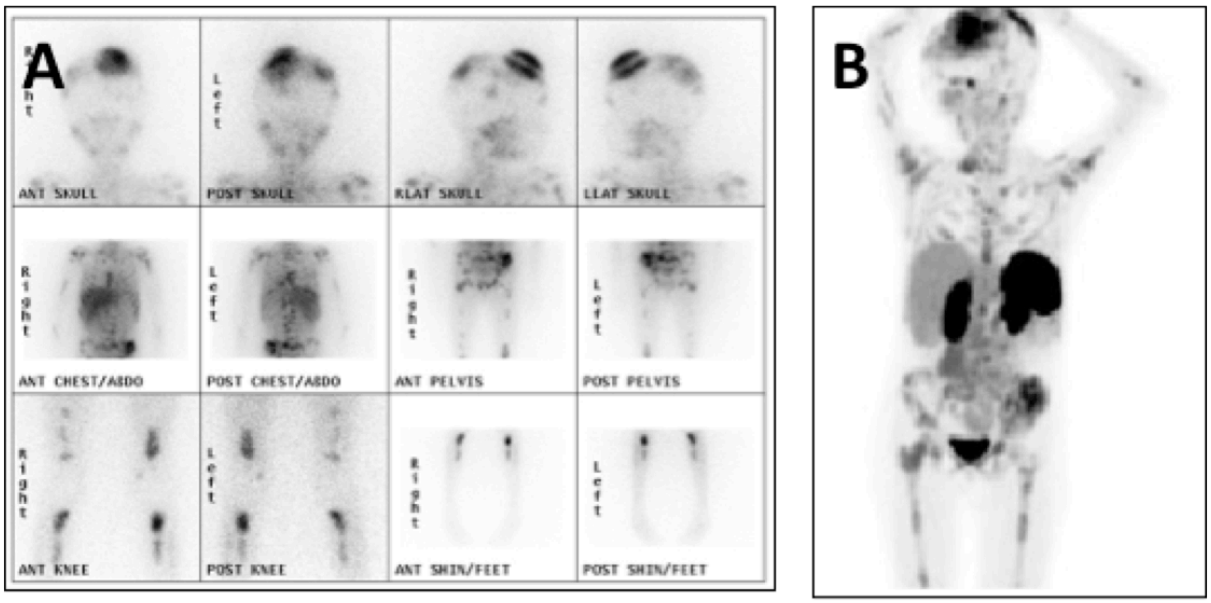


Figure 2.7 Patient 12: no skeletal deposits with uptake in the soft tissue mass only on ^{123}I-mIBG and ^{68}Ga-DOTATATE PET (score=1).

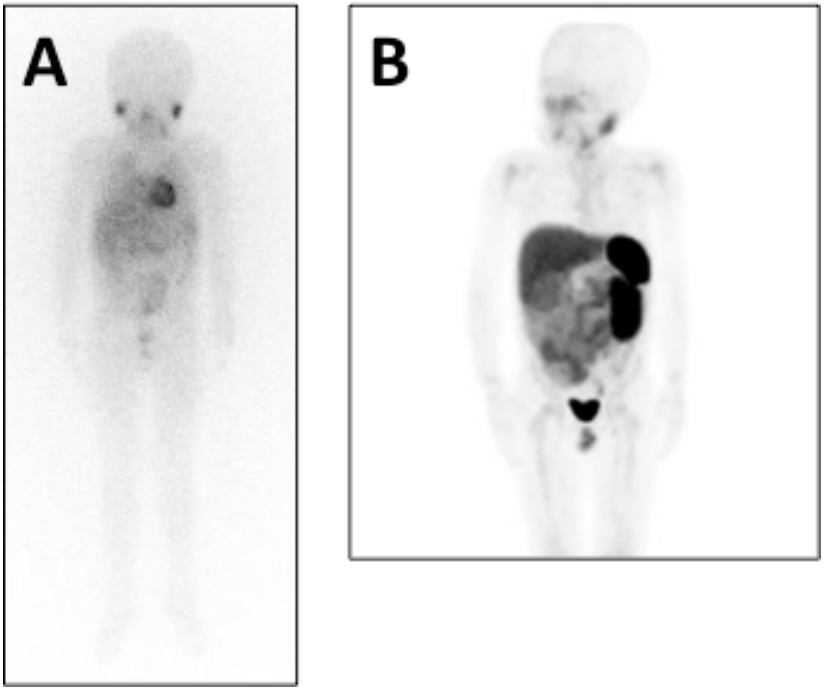


Figure 2.8 Patient 20: Greater SIOPEN skeletal score on ^{123}I-mIBG (score=24) (A) than on ^{68}Ga-DOTATATE PET (score=13) (B). The soft tissue score=1 for both imaging modalities.

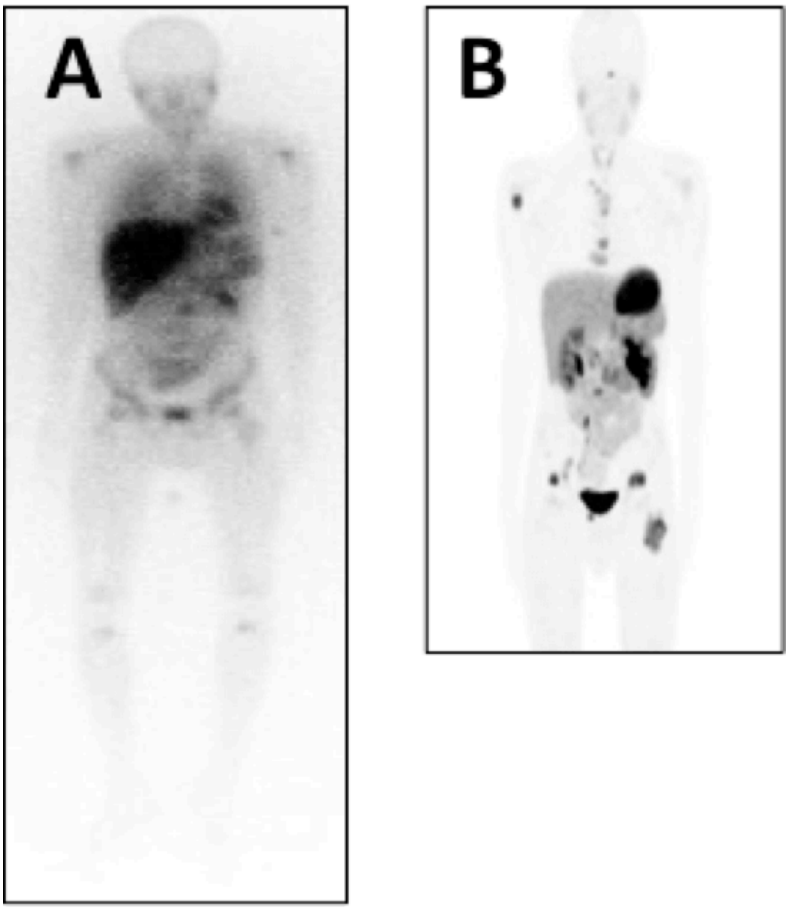


Figure 2.9 Patient 9: higher SIOPEN skeletal score on ^{123}I-mIBG (SIOPEN skeletal score = 6) (A) than ^{68}Ga-DOTATATE PET (SIOPEN skeletal score = 0) (B). The soft tissue score = 1 on both imaging modalities.

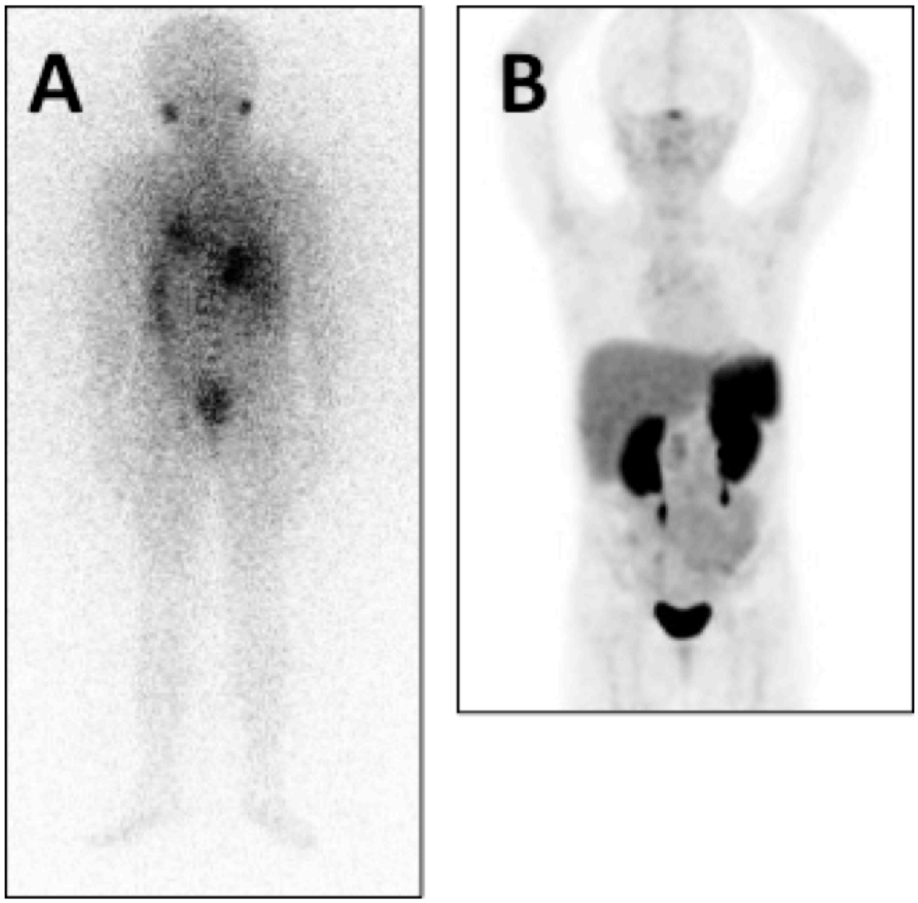


Figure 2.10 Patient 15. This patient had higher SIOPEN and soft tissue scores on ^{68}Ga-DOTATATE (SIOPEN skeletal score = 6) (B) than ^{123}I-mIBG (SIOPEN skeletal score = 0) (A). However, the soft tissue score was greater on the ^{123}ImIBG (soft tissue score = 2) than the ^{68}Ga-DOTATATE PET (soft tissue score=1).

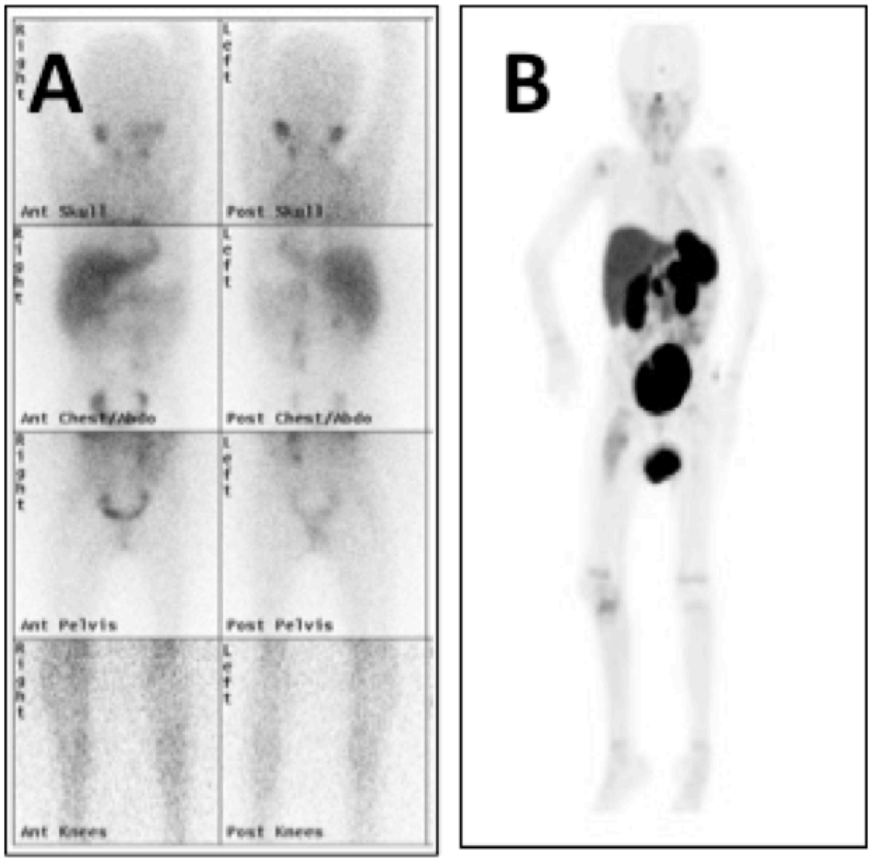


Figure 2.11 Patient 2. This patient had higher SIOPEN skeletal scores on ^{68}Ga-DOTATATE PET (SIOPEN skeletal score = 52) (B) than ^{123}I-mIBG (SIOPEN skeletal score = 23) (A). The soft tissue score = 0 on both imaging modalities.

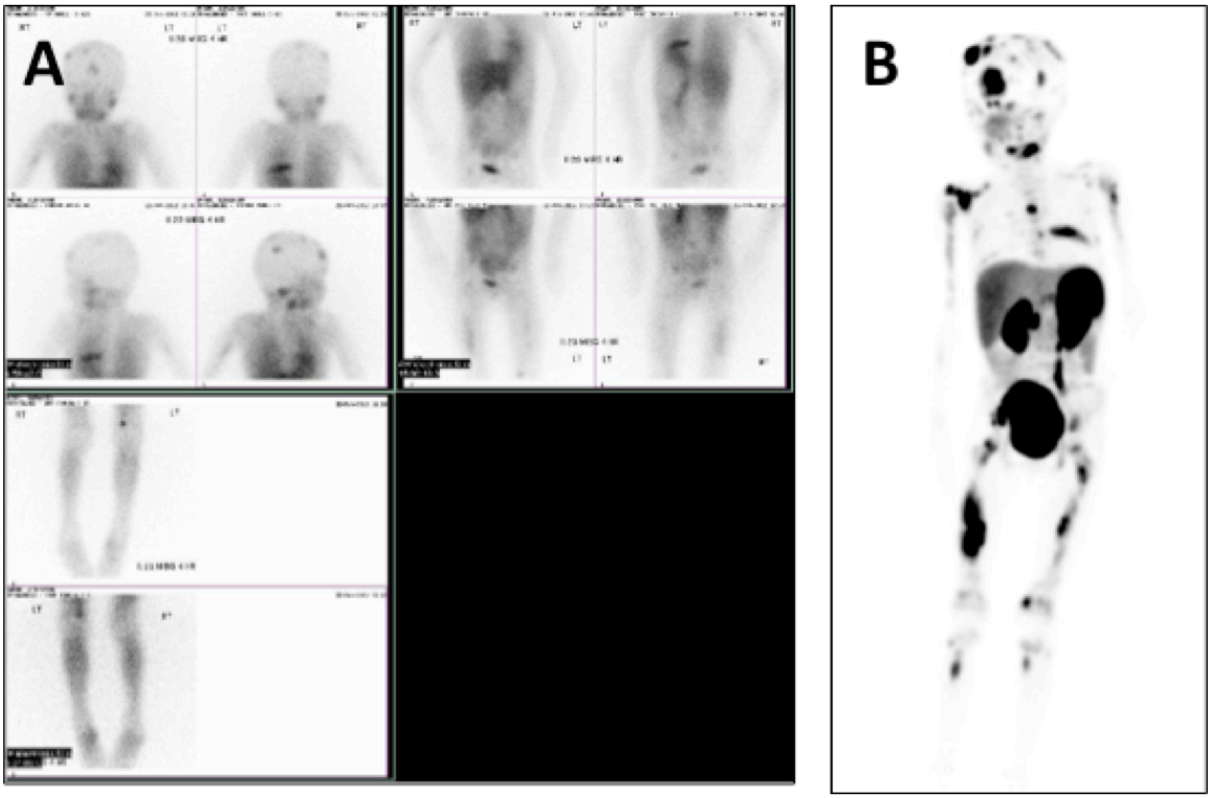


Figure 2.12: Patient 1. This patient had higher SIOPEN skeletal scores and soft tissue score on ^{68}Ga-DOTATATE PET (SIOPEN skeletal score = 35) (soft tissue score = 2)(B) than ^{123}I-mIBG (SIOPEN skeletal score = 8) (soft tissue score = 1)(A).

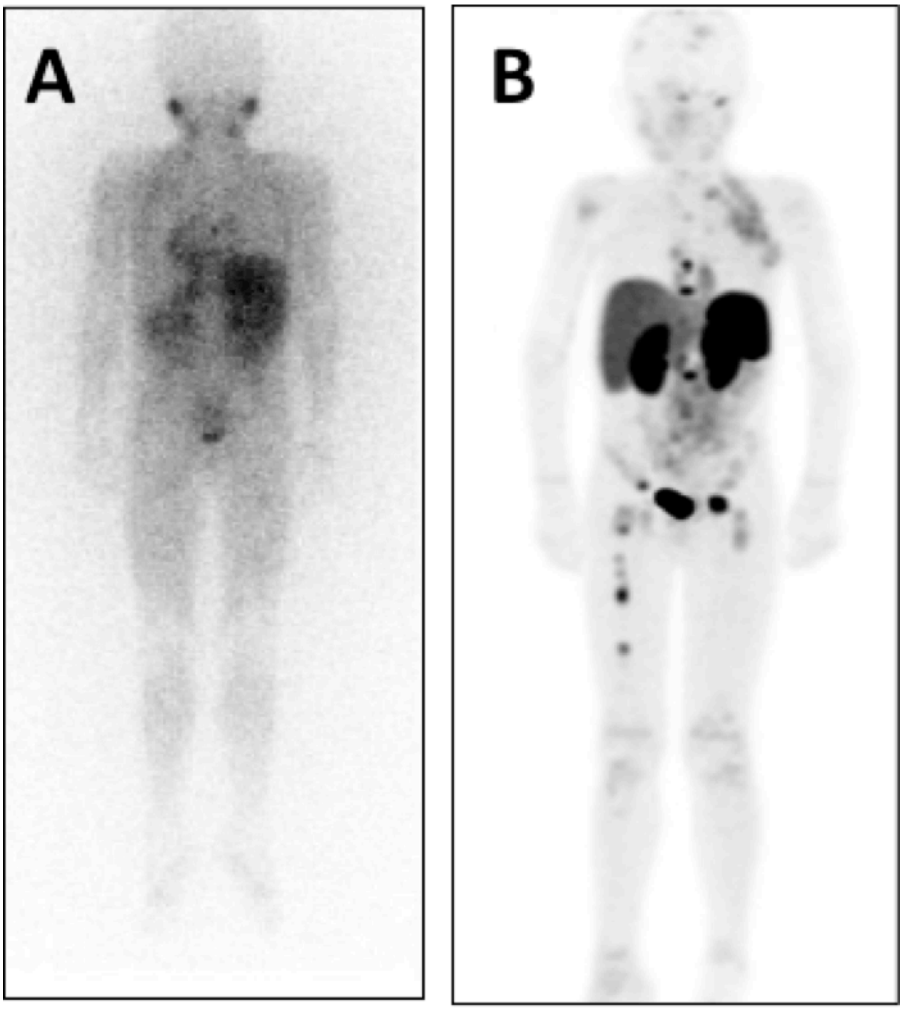
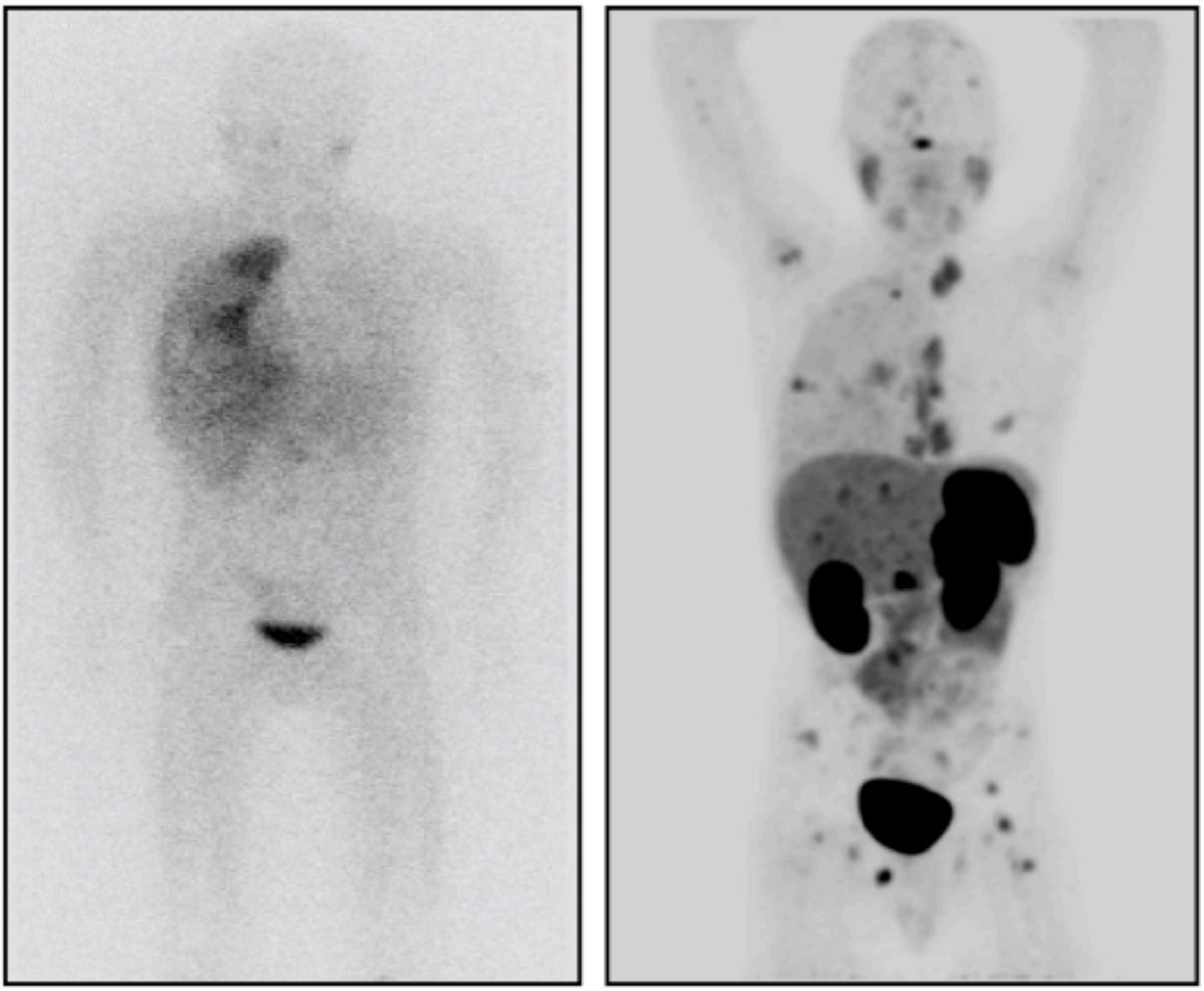


Figure 2.13: Patient had discordant uptake on the two imaging modalities. The ^{123}I-mIBG scan showing good uptake in the right hemithorax and no bone metastatases. The ^{68}Ga-DOTATATE scan showing multiple bone metastases and less uptake in the right hemithorax than the ^{123}I-mIBG.



2.3 ^{177}Lutetium DOTATATE molecular radiotherapy for neuroblastoma

The rationale for using ^{177}Lutetium DOTATATE in neuroblastoma include:

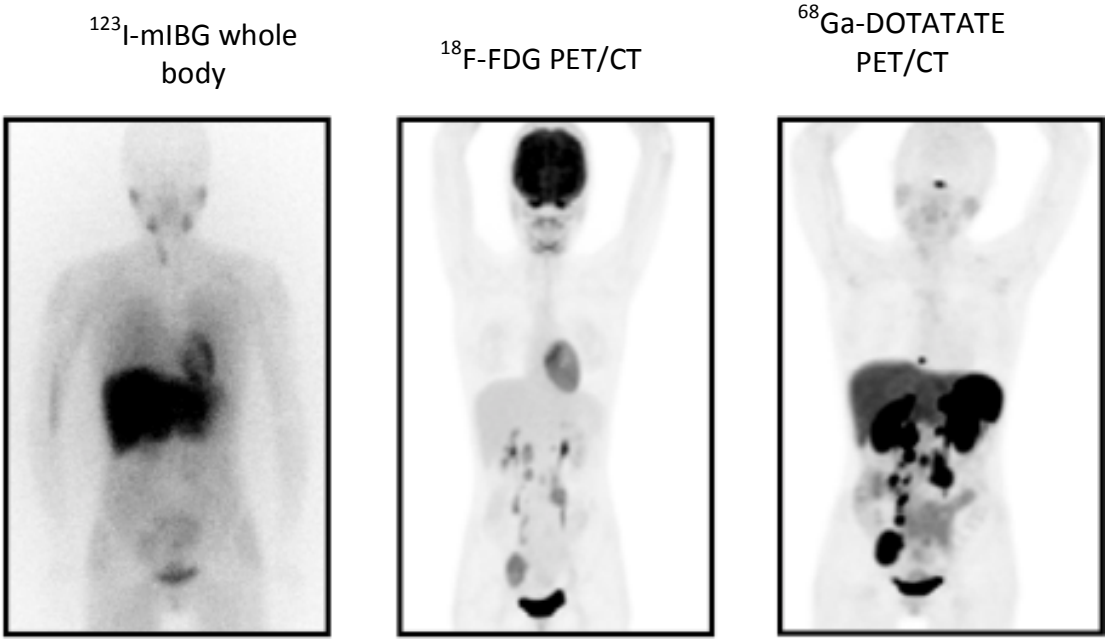
1. Neuroblastoma is a radiosensitive disease
2. The outcome for relapsed and refractory neuroblastoma is very poor and new therapeutic options are required
3. Molecular radiotherapy can target multiple tumour deposits with radiation from the same administration
4. SSTR2 receptors are frequently expressed by neuroblastomas
5. ^{177}Lutetium DOTATATE involves a distinct and separate cell surface molecular target to the noradrenaline transporter molecule targeted by mIBG
6. PRRT has been used with good response rates and low incidence of toxicity in adults with somatostatin positive neuroendocrine tumours

2.3.1 Materials and Methods

This initial pilot study has involved 13 patients with relapsed or refractory neuroblastoma, treated between 2008 and 2012, at University College London Hospitals NHS Foundation Trust. The first six patients in the pilot study were published by *Gains et al*. (*J Nuc Med 2011*).

All patients were required to have a ^{68}Ga-DOTATATE PET/CT prior to being selected for therapy. Only those with uptake in the tumour deposits equal or greater than the uptake in the liver were considered eligible for therapy with ^{177}Lu-DOTATATE (Figure 2.3)

Figure 2.14 Patient with relapsed neuroblastoma showing different uptake patterns on ^{123}I-mIBG imaging (no uptake), ^{18}F-FDG PET/CT (low grade uptake) and ^{68}Ga-DOTATATE PET/CT (avid uptake). This patient was considered a suitable candidate for ^{177}Lutetium DOTATATE with avid uptake of ^{68}Ga-DOTATATE in tumour deposits (SUV_{max} =) greater than the uptake in the liver (SUV_{max} =). This patient would not have been suitable for treatment with ^{131}I-mIBG molecular radiotherapy.



Other eligibility criteria were that they had not been treated with prior radiolabelled somatostatin analogues; had adequate haematological, renal and hepatic function; and an adequate performance status for treatment.

The parents or guardians, as well as comforters and carers, of each patient gave written informed consent for treatment. The patients in this pilot study were treated on a compassionate basis when no other treatment options were available.

Patients were admitted into a dedicated radionuclide inpatient treatment room for their ^{177}Lu-DOTATATE therapy. Patients were supported by specialised paediatric medical and nursing staff.

^{177}Lutetium was commercially obtained from IDB in Holland and the DOTATATE obtained from Erasmus University, Holland. The ^{177}Luteitum DOTATATE was then reconstituted in at the University College London Hospitals NHS Foundation Trust in-house radiopharmacy.

To prevent nausea or vomiting, Ondansetron was given twice daily orally for 5 days (5mg m-2; maximum 8mg) and dexamethasone (250µg kg-1) daily orally in 2 divided doses for 5 days.

Patients were given iv hydration with sodium chloride 0.9% (3L/24h/1.7m^2). To reduce the dose to the kidneys, an intravenous infusion of amino acids (2.5% l-lysine HCl and 2.5% L-arginine in water for injection) at the rate of 1Litre over 4hours was commenced 30 minutes prior to the infusion of the radiopharmaceutical.

^{177}Lu-DOTATATE was administered intravenously by a nuclear medicine physician via a second pump over 30 minutes.

The administered activity of ^{177}Lu-DOTATATE was planned to be a fixed administered activity of 7.4GBq but due to various problems with labelling and production it ranged from 2.56GBq to 8GBq (median 7GBq). Repeated administrations were planned at 8 to 10 week intervals up to a maximum of 4 administrations.

Whole-body planar scans and SPECT/CT scans were performed after treatment to examine uptake of the therapeutic agent (see Figure 2.). All acquisitions were performed on a GE Infinia Hawkeye 4 dual detector system. Whole-body planar scans were performed with an emission window of 208 keV ± 10%. Bed speed was 10 cm/min. SPECT/CT imaging was performed with an emission window of 208 keV ± 10%, with 120 projections and 30 s per projection, CT scanning was performed at 140 kVp and 2.5 mA, with a slice thickness of 5mm. Iterative reconstruction was used (20 subsets) with a 3D Butterworth filter and attenuation correction from the CT dataset.

Figure 2.15 – A pre-therapy ^{68}Ga-DOTATATE PET/CT MIP image (Fig. 2.15A) to document disease extent and confirm suitability for therapy; and whole body planar scans following administration of ^{177}Lu-DOTATATE. Post administration 1 (Fig 2.15B) and post administration 4 (Fig 2.15C) confirming uptake of the therapeutic agent in the neuroblastoma deposits identified on diagnostic imaging, with partial response to therapy demonstrated.

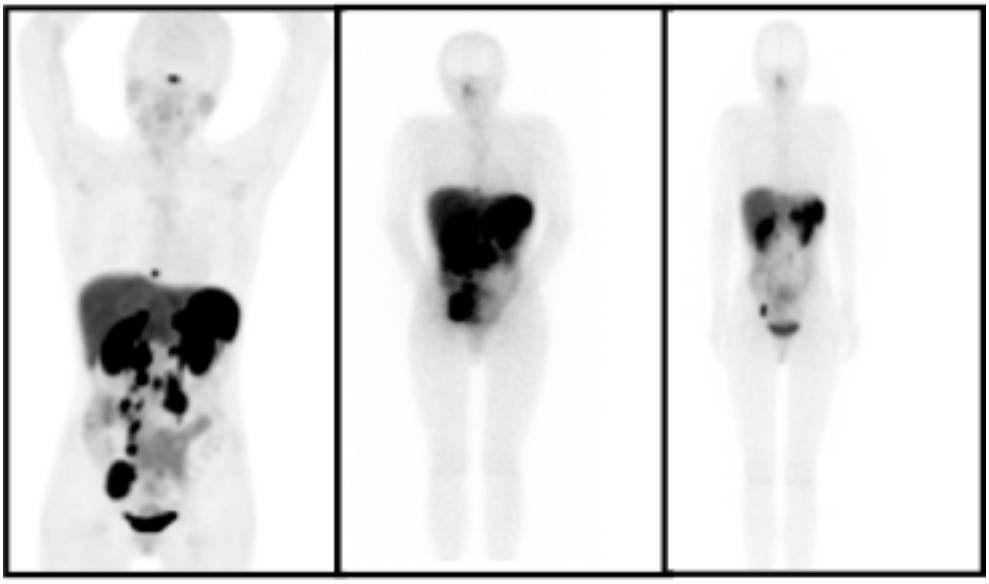


Figure 2.15A Figure 2.15B Figure 2.15C

Following administration, weekly full blood counts, urea and electrolytes and liver function tests were performed. Toxicity was graded using the Common Toxicity Criteria of the National Cancer Institute version 3.0. Patients were reviewed in the out-patient clinic 2 weeks after therapy, and re-assessment ^{68}Ga-DOTATATE PET/CT scans were performed at 6–8 weeks post treatment. ^{123}I-mIBG imaging and ^{18}F-FDG-PET imaging were also used in response assessment, but not all of the patients were imaged with all of the modalities. Pre- and post-treatment bone marrow aspiration and trephine were not routinely performed.

2.3.2 Results

Thirteen patients with neuroblastoma have been treated with ^{177}Lu-DOTATATE at University College London Hospitals NHS Foundation Trust between August 2008 and March 2013. 10 had relapsed neuroblastoma and 3 had refractory disease. 6 were male and 7 female. The median age was 8 years (range 2-23 years). 6 patients had had prior ^{131}I-mIBG molecular radiotherapy and 9 patients had had prior myeloablative therapy. All patients were treated on a compassionate basis when no other treatment options were available.

Table 2.4 Patient characteristics of those treated with ^{177}Lu-DOTATATE

Patient No	Age	M/F	Relapsed/ Refractory	Prior HD therapy	Prior ^{131}I-mIBG therapy
1	8	F	Relapsed	Yes	Yes
2	9	F	Relapsed	No	No
3	2	M	Refractory	Yes	Yes
4	6	M	Refractory	No	No
5	7	F	Relapsed	No	Yes
6	14	F	Refractory	No	Yes
7	9	M	Relapsed	Yes	Yes
8	8	F	Relapsed	Yes	Yes
9	23	F	Relapsed	Yes	No
10	5	M	Relapsed	Yes	No
11	7	M	Relapsed	Yes	No
12	8	M	Relapsed	Yes	No
13	10	F	Relapsed	Yes	No

Table 2.5 The number of cycles administered, administered activities of ^{177}Lu-DOTATATE (in GBq) and maximum renal and haematological toxicities encountered for all 13 patients treated with ^{177}Lu-DOTATATE molecular radiotherapy

Patient No	Cycles of LuDO	Administered Activities (GBq)	Grade 3 Haem Tox	Grade 4 Haem Tox	Grade 3 or 4 Renal Tox
1	2	7.4, 7.4	Post 2, plts	x	x
2	2	5.2, 7.3	-	Post 2, plts	x
3	3	7.2, 7.1, 7.4	*	*	x
4	3	7.4, 7.2, 7.1	No	x	x
5	2	7.4, 7.5	Post 2, plts	x	x
6	3	4.04, 7.4, 7.2	Post 3, plts	x	x
7	2	7.4, 7.6	x	x	x
8	1	2.56	x	x	x
9	4	7.4, 7.25, 6.21, 7.7	Post 3, Ne	x	x
10	4	7.2, 7.39, 7.4, 7.3	*	*	x
11	1	7.36	x	x	x
12	2	8, 7.6	x	Post 2, plts	x
13	1	7.75	*	*	x

* These patients were platelet dependent prior to ^{177}Lu-DOTATATE therapy.

Table 2.6 Radiological and clinical responses for 13 patients treated with ^{177}Lu-DOTATATE

Patient No	No of cycles	Response
1	2	Initial metabolic response; stable disease by RECIST; subsequent progressive disease
2	2	Initial metabolic response; stable disease by RECIST at 8 wk; subsequent progressive disease at 5 months
3	3	Partial metabolic response; stable disease by RECIST
4	3	Stable disease by RECIST; no metabolic response
5	2	Stable disease by RECIST; no significant metabolic response
6	3	Progressive disease
7	2	Progressive disease
8	1	Progressive disease
9	4	Partial response by RECIST; partial metabolic response
10	4	Clinical response with reduction in pain and improved performance status
11	1	Progressive disease
12	2	Progressive disease after 2 cycles
13	1	Progressive disease

Figure 2.16 – ^{68}Ga-DOTATATE PET/CT before and after 4 administrations of ^{177}Lu-DOTATATE (7.4GBq per administration). Whole body planar view (A and B) and axial slice through the main mass within the right psoas area. This patient had a partial response to treatment.

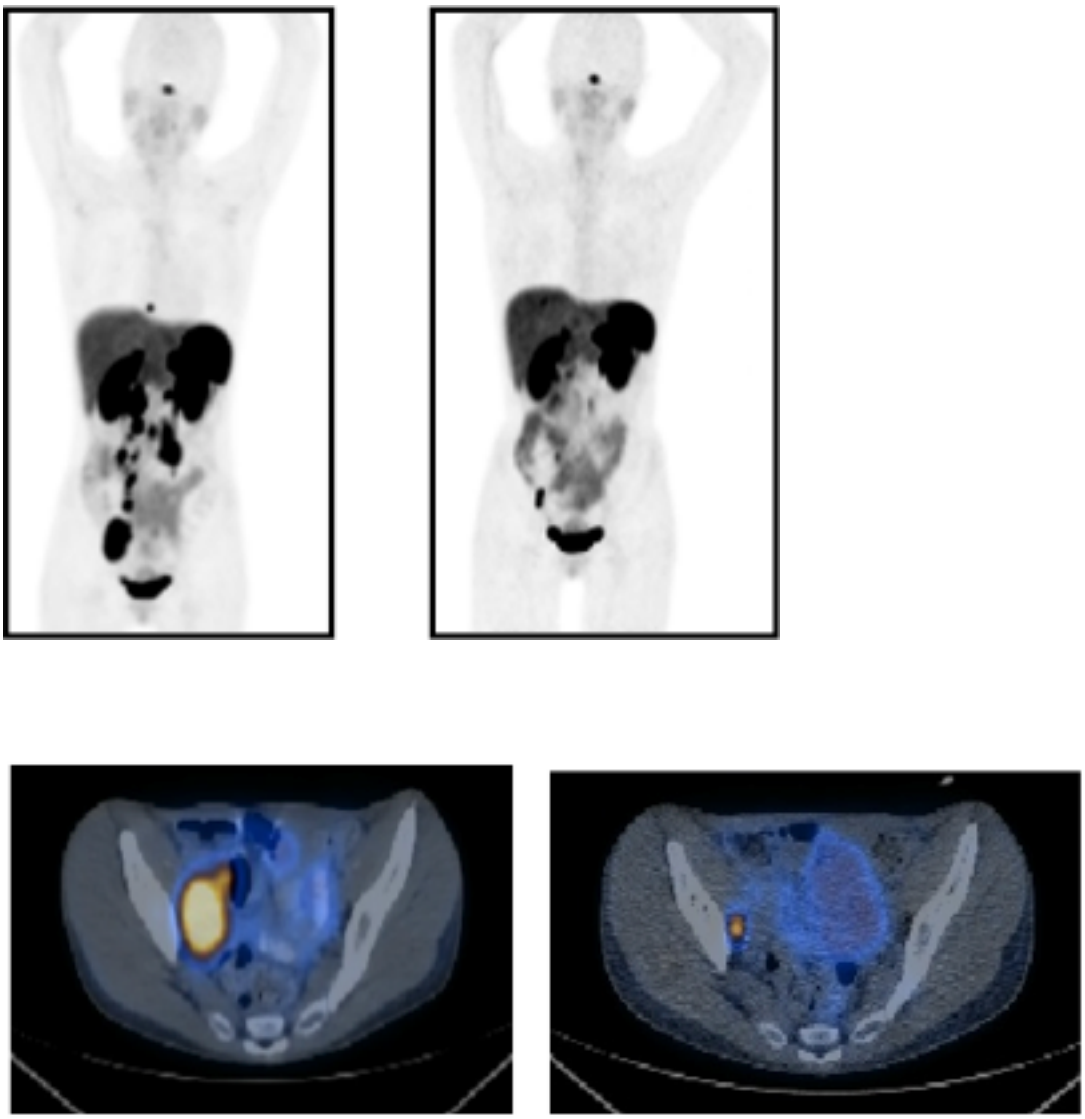
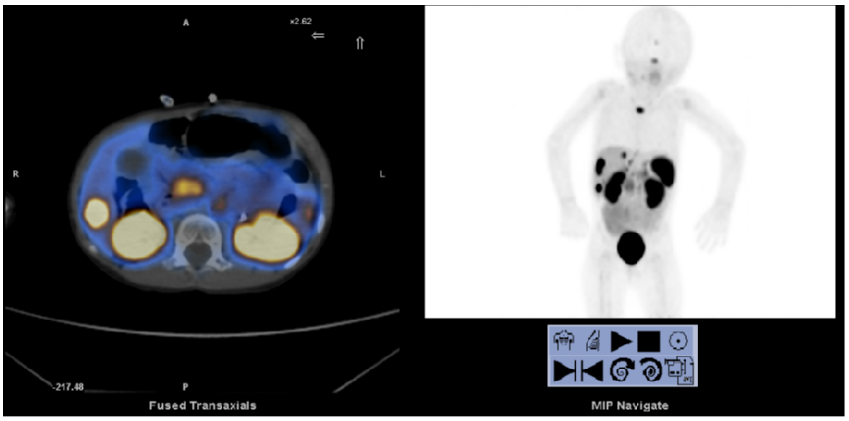
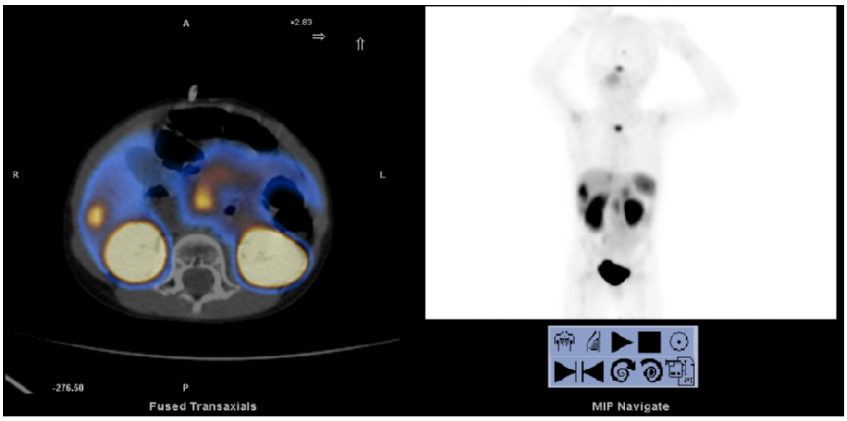


Figure 2.17

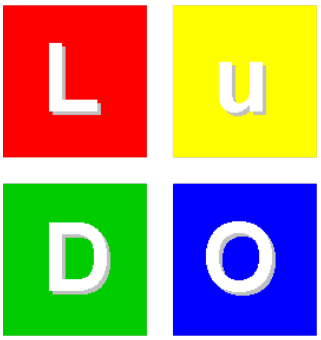
Pretreatment ^{68}Ga-DOTATATE PET/CT showing ^{68}Ga-DOTATATE-avid lesions in the T4 vertebral body and three metastases in the liver. Physiological uptake is seen in the pituitary, kidneys, bladder, stomach wall, liver and spleen. Repeat ^{68}Ga-DOTATATE PET/CT 1 year later post three administrations of ^{177}Lu-DOTATATE, showing a metabolic partial response with reduction in the SUV_{max} of the lesion in T4 (SUV_{max} reduced from 13.2 to 9.9) and the liver (lesion segment V, SUV_{max} reduced from 25.8 to 11) and no new lesions. Cross-sectional imaging showed a reduction in the size of the liver lesions although the patient had stable disease by RECIST criteria.





2.4 LuDO – a phase IIa trial of ^{177}Luetium DOTATATE in relapsed and refractory neuroblastoma

Following the demonstration on imaging with ^{68}Ga-DOTATATE PET/CT that patients with relapsed or refractory disease may benefit from therapy with radiolabelled somatostatin analogues, and promising data of safety and activity of ^{177}Lutetium DOTATATE in a pilot study, a phase IIa trial has been developed. The trial concept was initially developed by myself and Dr Mark Gaze at University College London Hospital NHS Foundation Trust. I had it accepted for presentation and development by myself at the joint Methods in Clinical Cancer Research, Jointly organised by ECCO, AACR, EORTC, ESMO in Flims, Switzerland in June 2010. The LuDO trial was then presented and approved by three of the NCRI clinical studies subgroups - CTRad, Neuroblastoma subgroup and Novel Agents subgroup and then by the main CCLG CSG. The trial was then taken on by the CRUK Clinical Trials Unit at the University of Birmingham for statistical input and preparation for the grant application to CTAAC, as well as submission to ethics ARSAC and Research and Development. The LuDO trial has now received funding through CRUK via CTAAC and opened to recruitment in October 2013. Four patients have been registered by June 2014.



The LuDO trial will be the first study to assess response rate, progression free survival and toxicity of ^{177}Lutetium DOTATATE in children with relapsed or primary refractory high risk neuroblastoma.

LuDO is a Phase IIa, open label, single centre, single arm Simon two stage design clinical trial. The primary objective is to evaluate the activity of ^{177}Lutetium DOTATATE in children with high risk relapsed or primary resistant neuroblastoma. The secondary objectives include describing the safety and adverse effects of ^{177}Lutetium DOTATATE in children with primary refractory or relapsed neuroblastoma, correlating tumour dosimetry (the absorbed dose in Gray) with response in patients treated with this regime, correlating somatostatin type 2 receptor (SSTR2) expression in the original histology with ^{68}Gallium DOTATATE PET/CT uptake and correlating the uptake on ^{68}Gallium DOTATATE PET/CT by SUVmax (maximum standardized uptake value) with response to ^{177}Lutetium DOTATATE therapy.

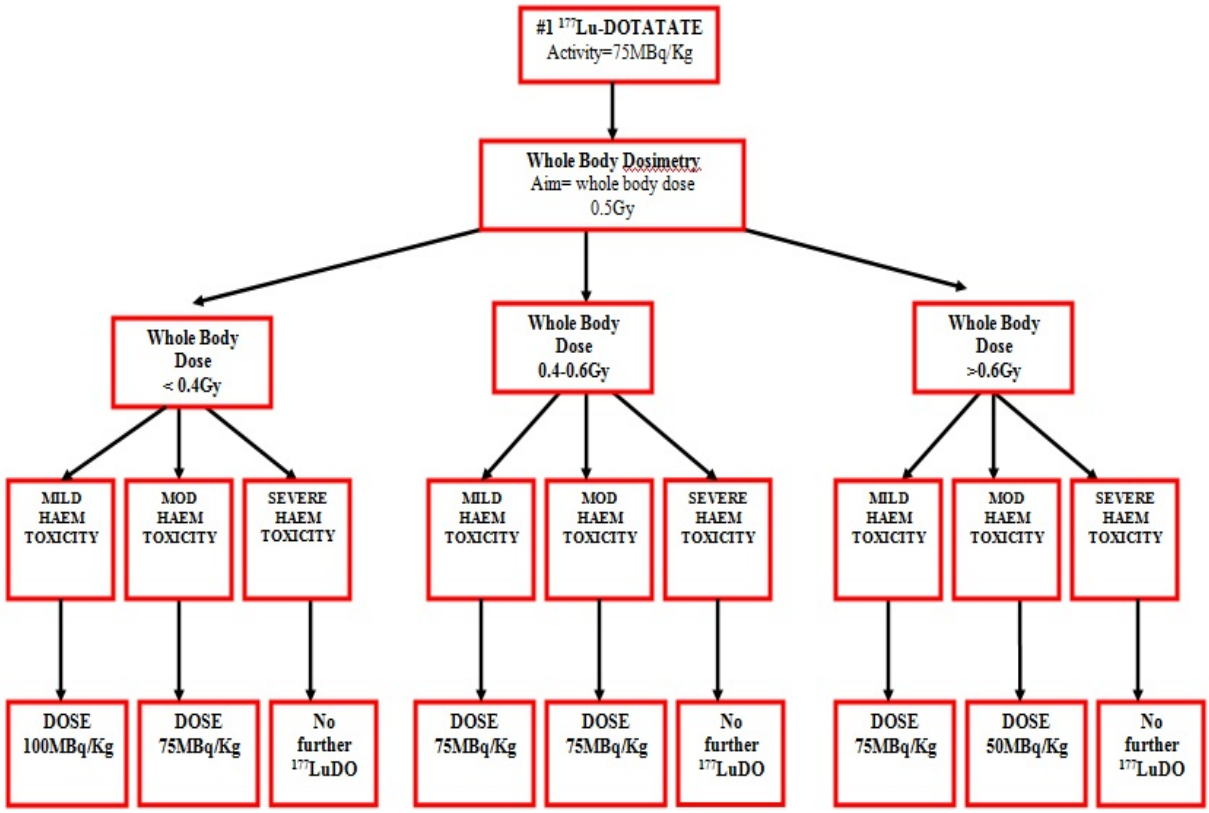
The trial will include children and young People > 18 months to < 18 years old with high risk, relapsed or primary refractory neuroblastoma (INSS stage 4 or INRG stage M). Children with high risk relapsed or refractory neuroblastoma will be assessed for suitability with ^{177}Lutetium DOTATATE by ^{68}Gallium DOTATATE PET/CT. Eligible patients must have uptake in the tumour deposits at least as high as the uptake in the liver.

Adequate dosimetry is essential for safe and effective treatment with ^{177}Lu-DOTATATE – the kidneys and the bone marrow being the dose limiting organs. There is an unpredictable relationship between the administered activity (in GBq) of a molecular radiotherapy agent and the absorbed dose (in Gy) to both the normal tissues and the tumour. Individualised

patient dosimetry is gaining International recognition as an essential part of treatment with molecular radiotherapy with the aim of improving the therapeutic index and minimising toxicity. This becomes of paramount importance in this paediatric population who have been heavily pre-treated and frequently have bone marrow involvement. Neuroblastoma patients are entirely different to the adult neuroendocrine tumour patient group in the amount of treatment that they have received, the frequency of bone marrow disease and their huge variations in size of the patients within the age group studied. Individual patient dosimetry will therefore form an essential part of the LuDO trial. This will enable the maximally tolerated administered activity to be delivered per patient whilst minimising toxicity. The dosimetry data from the trial will help inform other ^{177}Lutetium DOTATATE studies in adults and other potential somatostatin positive paediatric tumours.

The administered activity for the first cycle will be weight based. The second cycle administered activity will be dependent on the whole body dose (aiming for 0.5Gy per cycle whole body dose) and the haematological toxicity from the previous cycle as shown in Figure 2.18.

Figure 2.18 Flow chart describing the dosing of ^{177}Lu-DOTATATE within the LuDO trial



Patients will receive up to 4 administrations of ^{177}Lutetium DOTATATE. The statistical design is a Simons two-stage design. 14 patients will be treated in step 1 and if a response is seen in 4 out of the 14 patients we will move on to step 2 to recruit a further 10 patients. Therefore the total sample size for the trial is 24 patients.

The primary endpoint is response rate by International Neuroblastoma Response Criteria at 1 month after the completion of therapy. Secondary endpoints include progression free survival, overall survival and toxic effects and response rate by International Neuroblastoma Response Criteria at 4 months after the completion of therapy.

There are three translational studies within the trial. The tumour dose (in Gray) will be correlated with response to establish whether it is only those tumour deposits receiving a certain threshold dose that respond. Immunohistochemistry will be preformed on the original histopathology samples for somatostatin type 2 receptor (SSTR2) expression and then this will be correlated with the uptake on ^{68}Ga-DOTATATE PET/CT. The change in uptake on ^{68}Gallium DOTATATE PET/CT by SUV_{max} (maximum standardized uptake value) will also be correlated with response to ^{177}Lutetium DOTATATE therapy in the trial.

2.5 Discussion

This is the first reported evidence of the use, in children with neuroblastoma, of ^{68}Ga-DOTATATE PET/CT for therapy selection and response assessment and of ^{177}Lu-DOTATATE for molecular radiotherapy. The first six patients within the pilot study have been published in the *Journal of Nuclear Medicine* by Gains *et al. 2011.*

The established effectiveness of peptide receptor radionuclide imaging and therapy in adult neuroendocrine tumors provided the rationale to use this approach in children with neuroblastoma. PRRT in adults with somatostatin-positive neuroendocrine tumors has resulted in improved symptoms, prolonged survival, and an enhanced quality of life.

The use of ^{68}Ga-DOTATATE PET/CT in neuroblastoma has not been previously reported. There is one small study in the literature comparing ^{68}Ga-DOTATOC PET and ^{123}I-mIBG imaging in 5 female patients with neuroblastoma. The ^{68}Ga-DOTATOC PET was able to detect more lesions with a sensitivity of 97.2% ($p<0.0001$) compared to a sensitivity of 90.7% with ^{123}I-mIBG ($p<0.09$) (*Kroiss 2011*).

Menda et al. reported a phase I trial using 90Y-DOTATOC in children and young adults with tumors expressing somatostatin receptors, including two patients with neuroblastoma (*Menda 2010).* From their dosimetry data, they found the maximum tolerable dose of ^{90}Y-DOTATOC to be highly variable and proposed individualized dose administration as a preferred method to improve efficacy and reduce toxicity. ^{90}Y is a pure β-emitter, and therefore dosimetry and imaging have historically required concurrent administration of

^{111}In-DTPA-D-Phe1-octreotide whereas ^{177}Lu-DOTATATE emits γ- and β-radiation, allowing essential dosimetry measurements and imaging to be performed from the same administration. There have been reports on the feasibility of ^{90}Y PET/CT; the main limitation is the data acquisition time (*Walrand 2010, Werner 2010*).

A large amount of discordance was found in this cohort of patients between ^{68}Ga-DOTATATE and ^{123}I-mIBG imaging. Most of the patients in this cohort were imaged at relapse or with primary refractory disease and only a small number at diagnosis. As can be seen from Table 2.3, some patient's neuroblastoma deposits preferentially took up ^{123}I-mIBG and some preferentially ^{68}Ga-DOTATATE. This has implications for staging, response assessment and treatment selection in neuroblastoma. We have seen within the same patient inter-tumour heterogeneity with some tumour deposits preferentially taking up ^{123}I-mIBG and others ^{68}Gallium DOTATATE (see figure 2.13).

There are two contributing factors to the different uptake patterns on the ^{123}I-mIBG scan and the ^{68}Ga-DOTATATE PET/CT – a greater expression of SSTR2 than NAT by the tumour deposits and a greater spatial resolution for the ^{68}Ga-DOTATATE PET/CT. ^{124}I-mIBG PET is currently being examined in a clinical trial sponsored by the CRUK Drug Development Office and its potential greater spatial resolution will be examined. ^{68}Ga-DOTATATE PET/CT in neuroblastoma is still a research tool and its widespread use would need to be examined in a large number of patients on a multicentre basis ideally with patients being treated on a standard protocol or clinical trial and the limited availability of ^{68}Ga-DOTATATE PET/CT will currently limit this.

The heterogeneity of uptake on ^{123}I-mIBG and ^{68}Ga-DOTATATE PET/CT does lead to support for the use radiolabelled somatostatin analogues to be examined as a molecular radiotherapy treatment option for neuroblastoma patients as well as ^{131}I-mIBG. Within our institution, if a patient with relapsed or refractory neuroblastoma is referred for consideration of molecular radiotherapy we now image them with ^{123}I-mIBG and ^{68}Ga-DOTATATE PET/CT to fully map the extent of disease and to enable the selection of the most appropriate molecular radiotherapy agent for that individual. As has been shown above, within the same patient there is heterogeneity between deposits of neuroblastoma and this gives a good basis for possible future evaluation of combination radionuclide therapy.

Neuroblastoma patients with somatostatin receptor–positive disease identified with ^{68}Ga-DOTATATE PET/CT were deemed eligible for therapy with ^{177}Lu-DOTATATE. ^{177}Lu-DOTATATE offers a unique treatment target and differs from ^{131}I-mIBG and chemotherapy in its mode of action. Patients were considered eligible for therapy only if disease sites showed avid uptake of ^{68}Gallium DOTATATE equal or greater than the uptake in the liver. Patients with inadequate uptake by pre-therapy functional imaging are likely to represent tumour deposits with a poor expression of SSTR2 and these tumours would therefore not uptake sufficient ^{177}Lutetium DOTATATE to have a cytotoxic effect. The SIOPEN semi-quantitative scoring system used for assessment of both the ^{68}Ga-DOTATATE and ^{123}I-mIBG imaging assesses the number and location of areas of uptake. The intensity of the uptake is not taken into account. Therefore, even though only patients with uptake in tumour deposits greater or equal to the uptake in the liver on ^{68}Ga-DOTATATE PET would be suitable for therapy with ^{177}Lu-DOTATATE, ^{68}Ga-DOTATATE PET/CT is a good tool for imaging and response assessment of neuroblastoma.

^{177}Lu-DOTATATE is not proposed as a competitor to ^{131}I-mIBG but as a therapeutic option for those who have relapsed or have primary refractory disease and who may or may not have already received ^{131}I-mIBG therapy. A combination of the two agents may be more effective than either used alone. Although both cause myelosuppression, they may complement each other in being able to deliver targeted radiation to different areas of the tumor, depending on the molecular target expressed. We have seen clinically within our practice, inter-tumour heterogeneity in patients with neuroblastoma, with some tumour lesions taking up mIBG and some octreotate, therefore showing differential expression of NAT and SSTR2. We have also seen this on immunohistochemical analysis of both NAT and SSTR2 (see chapter 3).

Objective responses to ^{177}Lu-DOTATATE were seen in 4 patients a subjective response in one, and 2 further patients had stable disease. The patients treated were all heavily pre-treated and referred when no further treatment options were available and it is therefore not surprising that responses were not seen in all those cases. The treatment did not have a detrimental impact on quality of life for these children.

In studies of adults with neuroendocrine tumors, ^{177}Lu-DOTATATE has been well tolerated, with little acute toxicity. The dose-limiting toxicities were renal and hematologic. For adult neuroendocrine tumour patients, ^{177}Luteitum DOTATATE is often the first line of treatment and patients very rarely have bone marrow disease. None of the children in our study have developed any significant renal toxicity (although follow-up duration is short). The pilot study was feasible and the toxicity of ^{177}Luteitum DOTATATE was limited to haematological toxicity. The incidence of haematological toxicity is much greater than in the adult

neuroendocrine tumour population treated with ^{177}Lutetium DOTATATE. This is not surprising given that patients with neuroblastoma frequently have bone marrow disease and have been heavily pre-treated including high-dose myeloablative therapy and ^{131}I-mIBG therapy in many. The fixed administered activity of 7.4GBq used in adult studies of ^{177}Luteitum DOTATATE does not reflect the varying size of children and published reports on ^{177}Lutetium DOTATATE in adults include very little dosimetry on either tumour or organ at risk doses.

All of the patients in this study had end-stage disease with few treatment options available and a poor prognosis. We aimed principally to assess ^{177}Lu-DOTATATE as a feasible therapy, not its response. When disease was measurable, RECIST were used. Disease that was evaluable but not measurable was assessed on ^{123}I-mIBG scans or ^{68}Ga-DOTATATE PET/CT. An account of the metabolic response is also given, but we acknowledge that this is not yet a validated approach.

As discussed in chapter 1, the standard method of response assessment for metastatic disease in neuroblastoma is with ^{123}I-mIBG. Many of the patients in this pilot study did not have a ^{123}I-mIBG scan for assessment of response as they were being treated on a compassionate basis and we wanted to minimise the imaging the patients had to undergo at this stage. ^{123}I-mIBG imaging would be problematic in those patients who have mIBG negative disease and in those patients who have heterogenous uptake with mIBG negative areas of disease.

It is not known whether a metabolic response on ^{68}Ga-DOTATATE PET/CT corresponds with an improved outcome for the patient and this is something that will be explored as part of the LuDo trial. Within the adult neuroendocrine tumour population, who have been treated with PRRT, decreased ^{68}Ga-DOTATATE uptake in tumours after the first cycle was able to predict time to progression and improvement in clinical symptoms (*Haug 2010*). Therefore I chose to report the metabolic response on ^{68}Ga-DOTATATE PET/CT as well as response as per RECIST criteria.

Dosimetry for PRRT is still developing (*Forrer 2005, Cremonesi 2010*) and will become an essential part of therapy with peptide receptor radionuclides because the same administered activity results in a variable absorbed radiation dose in Gray to both the tumour and the dose-limiting organs at risk, especially the kidneys and bone marrow. Individual patient dosimetry becomes even more important in the pediatric population, in which there is a greater variation in body surface area, potentially requiring a modification of the administered activity, and individuals cured of their cancer may live for decades with late effects. Individualised dosing will be performed based on whole body dose measurements and haematological toxicity within the LuDO trial. Tumour and organ at risk doses will also be calculated. This data will be essential to the development of future paediatric molecular radiotherapy trials.

If ^{177}Lutetium DOTATATE is shown to be effective, future developments could include potential combination therapy with ^{131}I-mIBG, dose escalation with stem cell support or the addition of radiosensitisers.

Chapter 3

Immunohistochemical evaluation of target expression in high-risk neuroblastoma tissue samples to facilitate optimisation of molecular radiotherapy

3.1 Introduction

Molecular radiotherapy targeting the noradrenaline transporter (NAT) molecule with *meta*iodobenylguanidine (mIBG) and the somatostatin receptor subtype-2 (SSTR2) with ^{177}Lutetium DOTATATE are therapeutic options for patients with relapsed and or refractory high-risk neuroblastoma (see chapters 2 and 4).

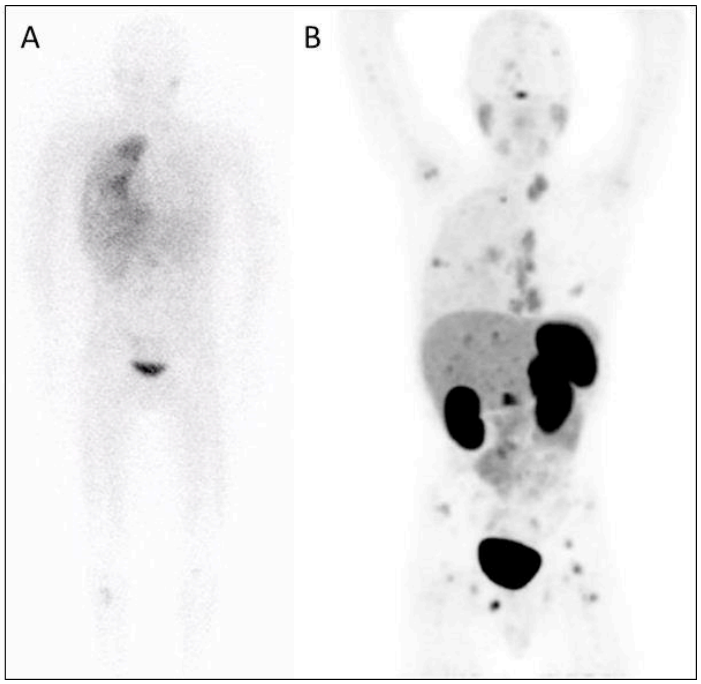
mIBG plays an important role in the management of neuroblastoma as previously discussed. ^{123}I labelled mIBG is essential for the diagnosis, staging and response assessment of neuroblastoma. ^{131}I labelled mIBG is an important therapeutic option for patients with relapsed or refractory disease. Response rates reported however, are very variable between the studies reported (see chapter 4). mIBG is primarily taken up by the noradrenaline transporter molecule (NAT) encoded by the SLC6A2 gene (*Glowniak 1993*).

^{68}Ga-DOTATATE and ^{177}Lutetium DOTATATE are both radiolabelled somatostatin analogues targeting SSTR2 expressed by many neuroblastomas, one used for imaging and the other for therapy (see chapter 2). Somatostatin is a 14 or 28 amino acid peptide found throughout the body. It works by the suppression of various peptide hormones including pancreatic secretion, neurotransmission and gut motility. Somatostatin acts on its various tissues mediated by the five known somatostatin receptors – SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5. SSTR2 is encoded for by chromosome 17 and is found within various normal tissues including pancreatic islet cells, kidney, stomach, duodenum and pituitary (*Taniyama 2005*). SSTR2 has been

found to be present in many neuroendocrine tumours and radiolabelled somatostatin analogues targeted against the SSTR2 have been used for imaging and therapy (see chapter 2). Neuroblastomas have also been found to have a high expression of SSTR2 (*Albers 2000*). Georgantzi et al. looked at the expression of the 5 different somatostatin receptor subtypes (SSTR1-5) by immunohistochemistry of formalin fixed paraffin embedded tissue from 11 children with neuroblastoma stages II-IV (9 of them stage IV). The most frequently expressed subtype was SSTR2, which was present in 90% of samples. SSTR4 was the least frequently expressed in only 21%, with the others showing reasonable expression SSTR5 in 79%, SSTR1 74% and SSTR3 in 68%. The expression did not change significantly with treatment. They also tested SSTR expression in 5 neuroblastoma cell lines and found variable expression (*Georgantzi 2011*).

DOTATATE and mIBG therefore have distinct and separate molecular targets. As has been shown in chapter 2, there is a significant variability and heterogeneity in the uptake on functional imaging of the two agents amongst neuroblastoma patients. We have observed inter-tumour variability on imaging with some patients having discordant uptake patterns and disease distributions on imaging with some lesions predominantly taking up mIBG and others DOTATATE (Figure 3.1).

Figure 3.1 Heterogeneity of uptake of ^{123}I-mIBG (Figure 3.1A) compared to ^{68}Ga-DOTATATE (Figure 3.1B) in the same patient.



Monoclonal antibodies directed against SSTR2 and NAT for immunohistochemistry are now commercially available. This study examines whether there is the same degree of heterogeneity in protein expression for NAT and SSTR2 as seen on functional imaging and also examines whether the degree of expression is of prognostic significance. The receptor expression of NAT and SSTR2 are examined across an unselected range of archival neuroblastoma tissue samples to allow correlation with clinical parameters and refine understanding of the targets used for molecular radiotherapy.

3.2 Material and Methods

Archived formalin fixed paraffin embedded (FFPE) neuroblastoma tissue samples were received from the Children's Cancer and Leukaemia Group tissue bank. Immunostaining was performed on an immunohistochemistry, Leica Bond-Max machine. Commercially available antibodies for NAT and SSTR2 were used in this study. For NAT, a monoclonal antibody, was obtained from Merck Millipore, Billerica, Massachusetts (catalogue number MAB5620). For SSTR2, a monoclonal antibody, UMB1, produced by Epitomics, distributed by Insight Biotechnology, Wembley, UK (catalogue number 3582-1 AC-0162RUO) was used. The antibodies underwent in house checking with positive and negative control tissues. This was adrenal medulla for the NAT antibody and pancreatic tissue for the SSTR2.

The FFPE slides were baked at 60°c for 1 hour then de-waxed and rehydrated through graded alcohol solution. A peroxidase block was then applied to the slide for 5 minutes. The primary antibody was then applied. For SSTR2 a dilution of 1:200 was applied for 15 minutes using heat induced epitope retrieval (HIER). For the NAT a dilution of 1:1000 was used. Post primary for 8 minutes. A polymer was applied for a further 8 minutes and DAB for 10 minutes. Haematoxylin was applied for a further 5 minutes.

Each slide was examined by myself and Neil Sebire, Professor of Paediatric and Developmental Pathology at The Institute of Child Health, University College London,

at Great Ormond Street Hospital NHS Foundation Trust. At the time of scoring, we were blinded to information on stage, age and mycN status of the patient and blinded to the results of the other immunostaining. The samples were categorised on morphology into differentiating, poorly differentiated and undifferentiated neuroblastomas.

Each sample was then semi-quantitatively scored for both of the antibodies (NAT and SSTR2) for

1. Percentage cells staining with the antibody (0-100%)
2. The intensity of expression of the antibody (0-100%)

These scores were estimated to the nearest 10%. For the analysis, a composite score combining the percentage cells staining and the intensity of expression was used (Dub*ois 2012, Volante 2007*).

The choice of cut-off points for categorising NAT and STTR2 is not clear in neuroblastoma and the prognostic influence of NAT and STTR2 measures is unclear, therefore analysis of measures in their original form (continuous, may take values between zero and 100) was performed. Categorising a continuous measure results in loss of information and power. The results of IHC staining from NAT and SSTR2 were linked with known available data available from the CCLG tissue bank on age, stage, MYCN amplification and outcome.

Data was summarised using summary statistics (mean, standard deviation, medians, range and proportions). Overall survival was calculated from the date of diagnosis to date of death or date last seen for the alive patients. A Kaplan-Meier curve is presented for survival data. One, two and three year survival rates are shown with 95% confidence intervals (CI). Cox proportional hazards model used to compare groups and calculate hazard ratios (HR). Martingale residuals plotted to verify linear assumption of NAT and SSTR2 in the model. Stata v12 was used for the calculations.

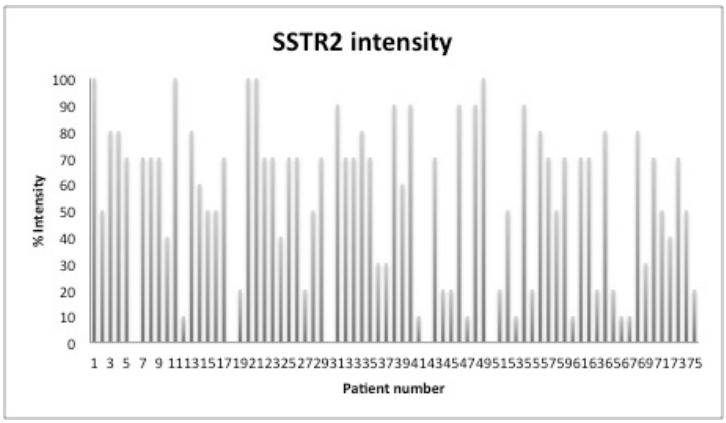
3.3 Results

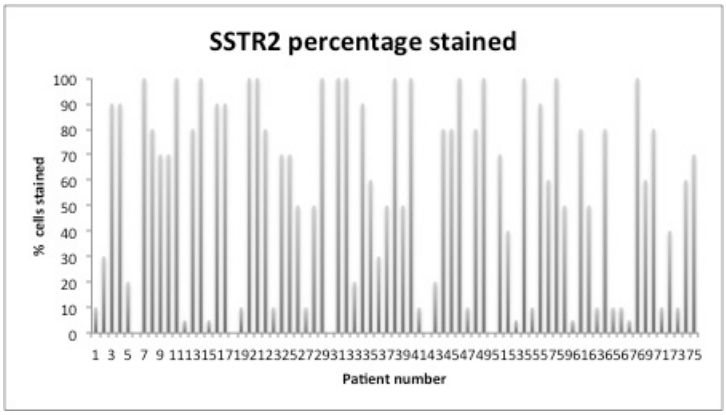
In total, 100 tissue samples were received from the CCLG tissue bank. 11 patients were excluded from the analysis – Wilms tumour (n=2), completely necrotic sample (n=1), normal tissue (n=4), ganglioneuroblastoma (n=4). For 10 patients, two or more samples were sent. In these cases, the earliest tumour sample was used for the statistical analysis but, the cases with multiple samples are reported separately to see if receptor expression changed with treatment (See Table 3.1).

75 samples were available for statistical analysis. The majority of the samples were poorly differentiated neuroblastoma (n=62), with the others being characterised on morphology as differentiating (n=10) and undifferentiated (n=3).

Marked variation in expression was seen between patients in individual tumour samples for both the intensity and percentage expression of cells as shown in Figures 3.2 and 3.3.

Figure 3.2 Histograms showing range of percentage intensity staining of cells (A), percentage number of cells stained (B) and the range of composite scores (C) for SSTR2.





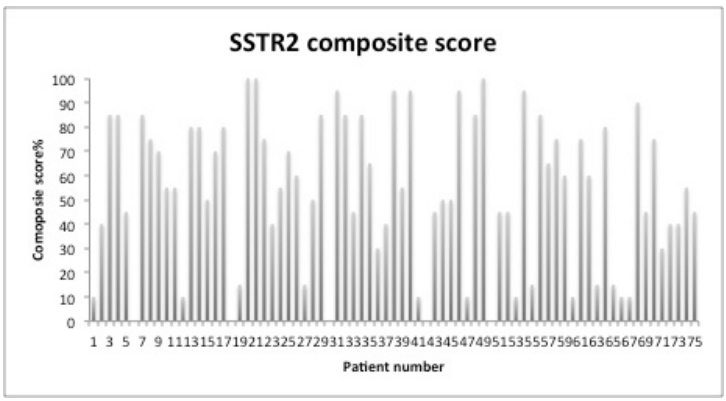
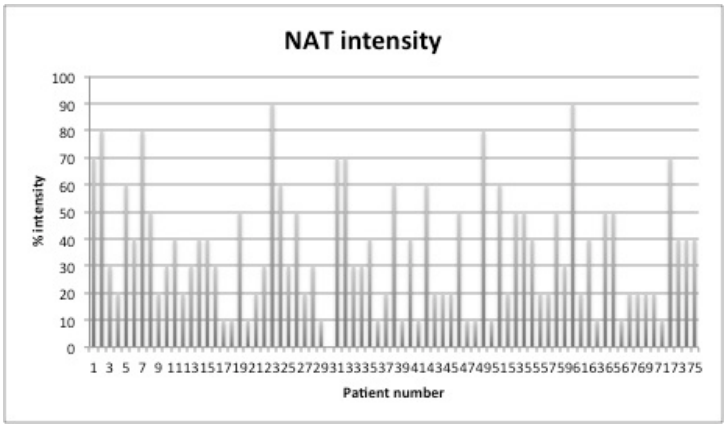
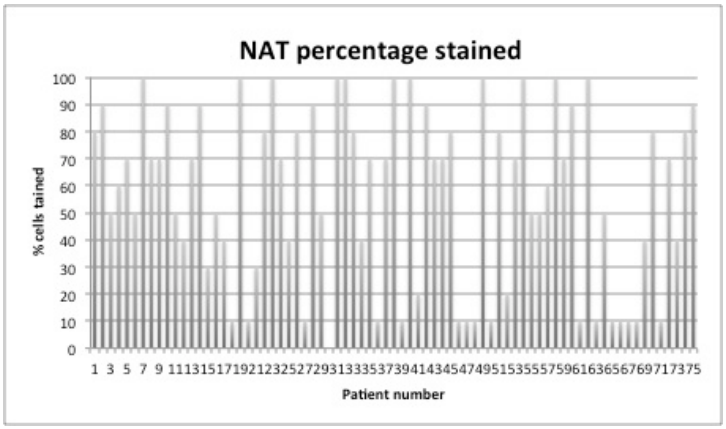


Figure 3.3 Histograms showing range of percentage intensity staining of cells (A), percentage number of cells stained (B) and the range of composite scores (C) for NAT.





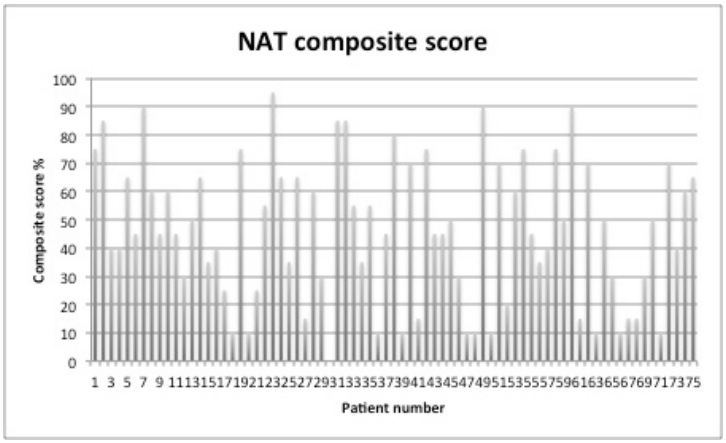


Figure 3.4A

Example showing change in receptor expression with treatment from poorly differentiated to differentiated. A (NAT 20% expression, 20% intensity) B (SSTR2 40% expression, intensity 30%) in poorly differentiated neuroblastoma. C (NAT expression 80% expression, 20% intensity) and D (SSTR2 100% expression and 90% intensity) in differentiated neuroblastoma.

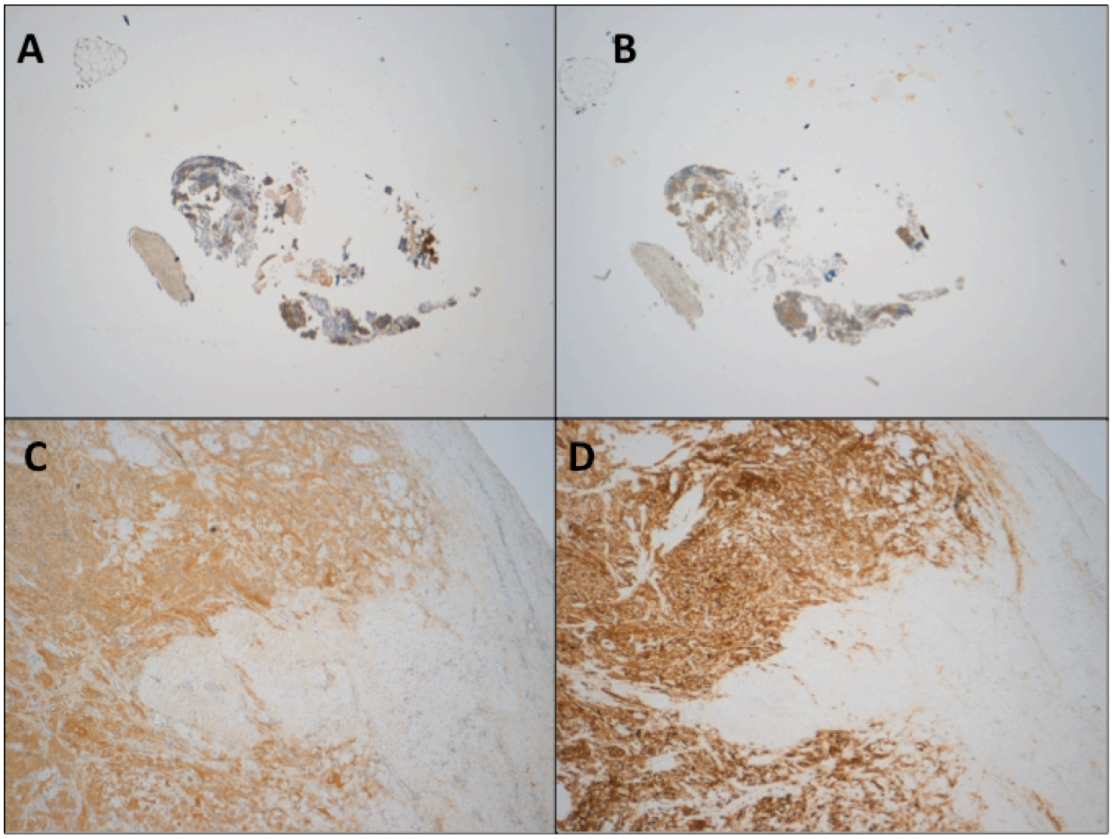


Figure 3.4B

Example showing intense staining with SSTR2 (A) (100% of cells with 100% intensity) compared to weak staining for NAT (B) (30% of cells with only 20% intensity) in the same part of a tumour.

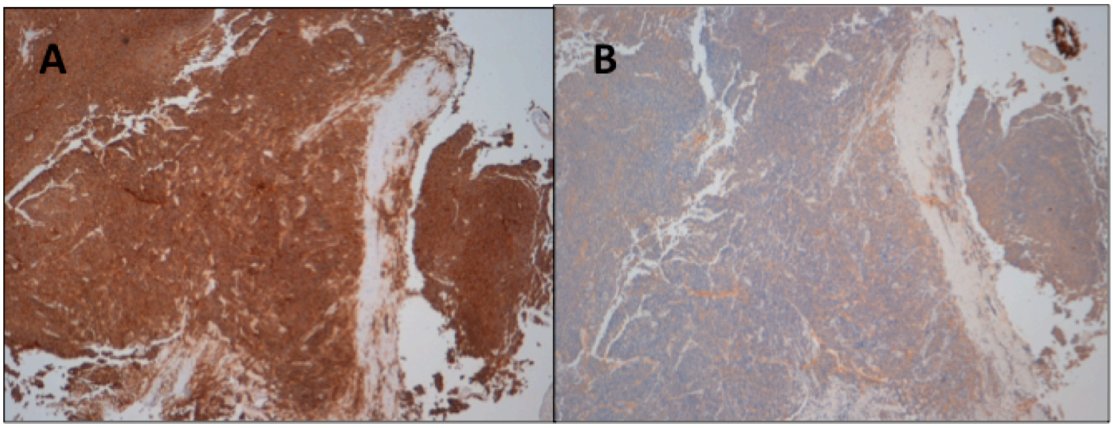


Figure 3.4C Example of variability of staining within a tumour sample. A (H and E stain), B (NAT staining), C (SSTR2 staining). In this case the NAT and SSTR2 staining showed the same pattern of distribution.

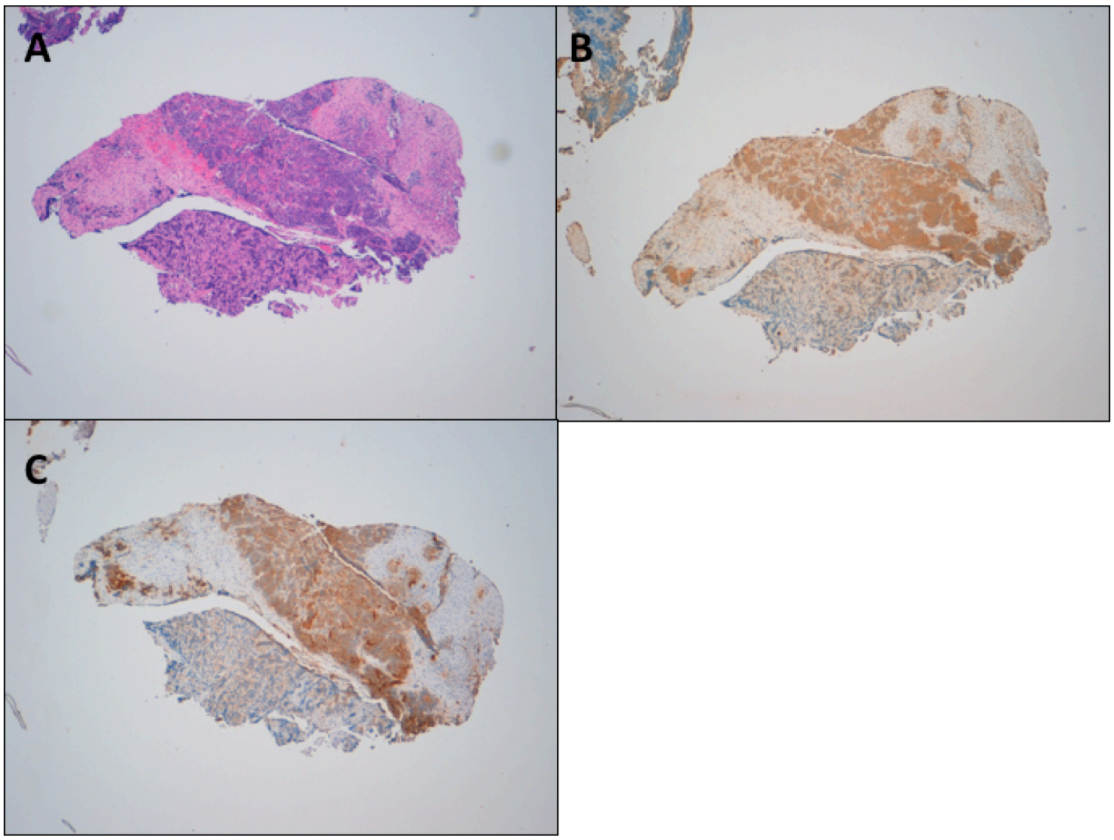


Figure 3.4D

Further illustration of variable staining pattern within a tumour. There are areas of high and low expression of the SSTR2 and NAT corresponding to areas of well and poorly differentiated neuroblastoma on H and E staining [H and E (A), SSTR2 (B), NAT (C)].

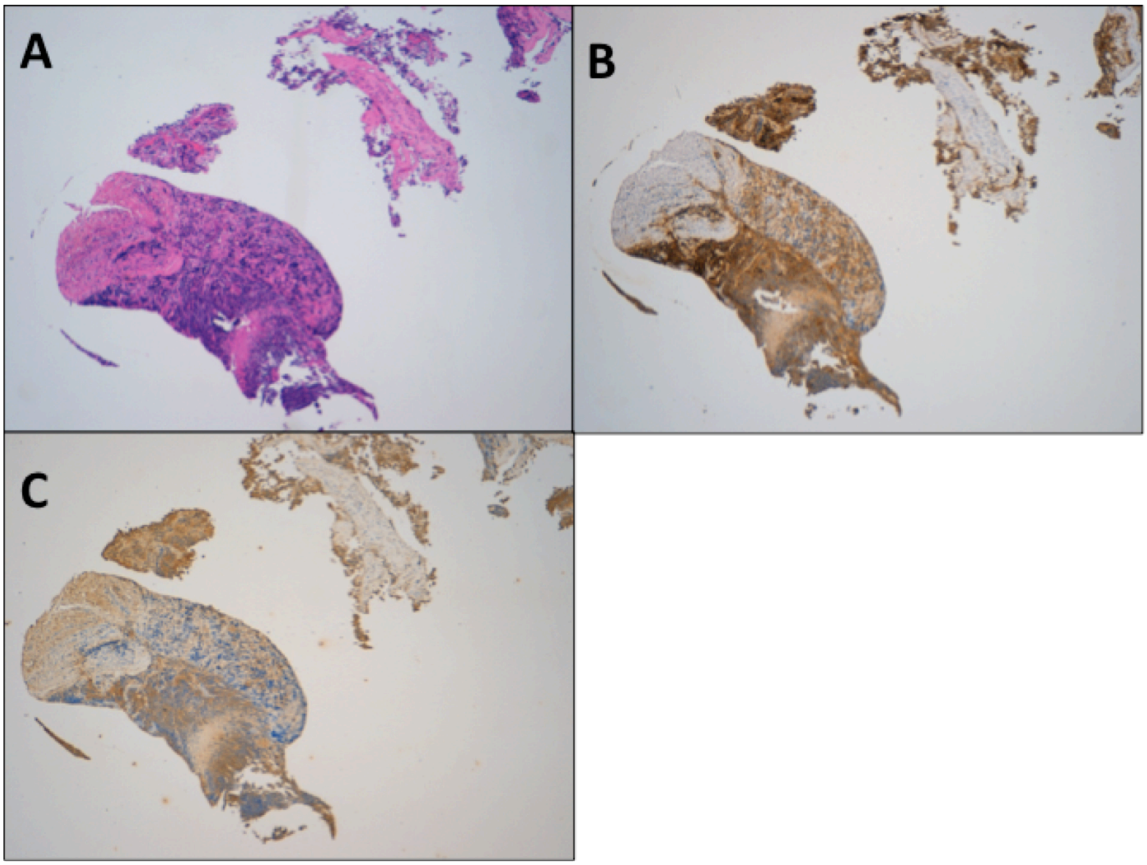
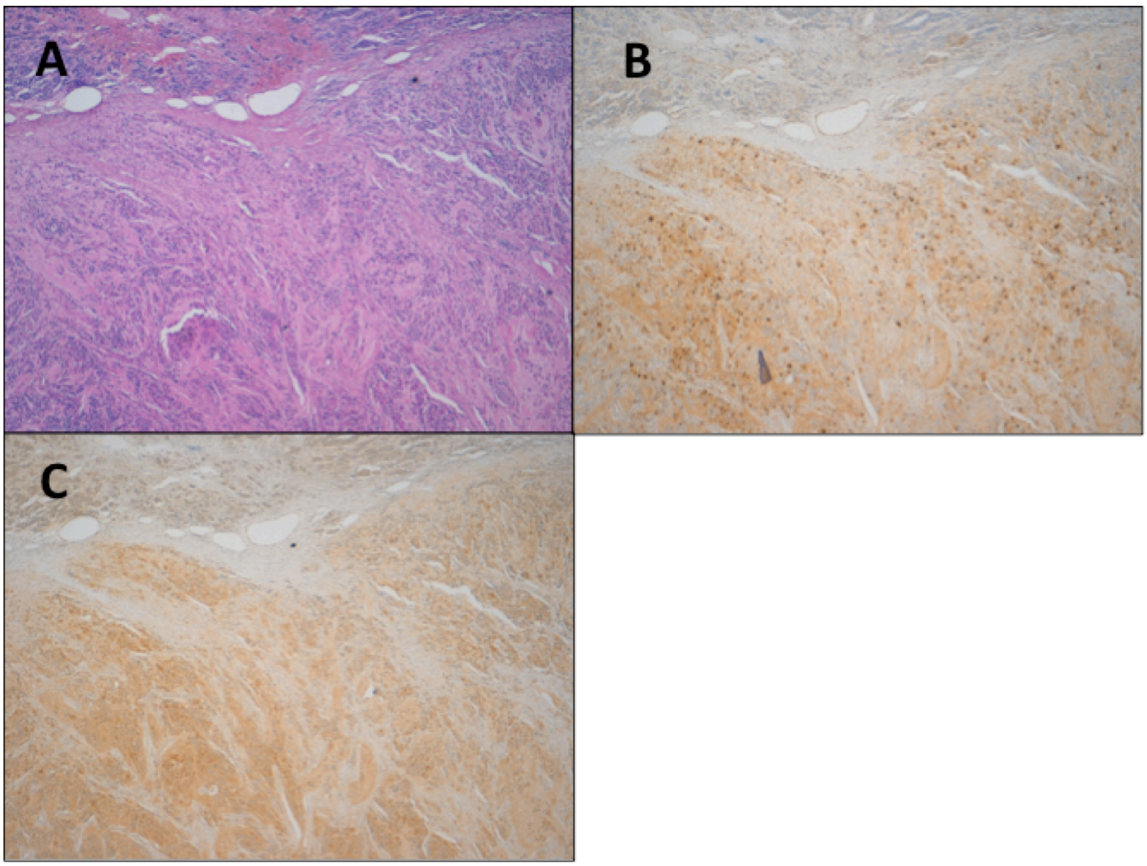


Figure 3.4E Example of staining with both SSTR2 (B) and NAT (C) compared to the H and E sample (A). The antibody staining picks out the ganglion cell differentiation within the tumour (more obviously seen on the SSTR2 staining than the NAT staining).



Where multiple samples were received for the same patient, the percentage change in expression of SSTR2 increased as the tumours changed with treatment from poorly differentiated to differentiated in all cases (n=5) compared to 4 out of the 5 cases for NAT.

Table 3.1 The change in expression of NAT and SSTR2 with treatment in cases where multiple samples were sent.

	Pathological Grading	SSTR2 % cells	SSTR2 intensity %	SSTR2 composite score	NAT % cells	NAT intensity %	NAT composite score
Example 1							
Sample 1A	Poorly differentiated	70	70	70	40	30	35
Sample 1B	Differentiating	100	70	85	100	50	75
Sample 1C	Differentiating	100	70	85	100	50	75
Example 2							
Sample 2A	Poorly differentiated	80	90	85	10	10	10
Sample 2B	Poorly differentiated	40	30	35	20	20	20
Sample 2C	Differentiating	100	90	95	80	20	60
Example 3							
Sample 3A	Poorly differentiated	90	80	85	60	20	40
Sample 3B	Differentiating	100	70	85	80	30	55
Example 4							
Sample 4A	Poorly Differentiated	50	50	50	30	30	30
Sample 4B	Differentiating	100	90	95	90	40	65
Example 5							
Sample 5A	Poorly Differentiated	50	50	50	90	30	60
Sample 5B	Differentiating	100	90	95	30	40	35

There was no correlation between the expression of NAT and of SSTR2 for all patients and when stage 4 only patients were examined. Figure 3.4A shows no correlation between NAT and SSTR2 for all patients (correlation = 0.23), and Figure 3.4B shows that there was no correlation between the receptors when just the stage 4 patients were examined.

Figure 3.5A Correlation between SSTR2 and NAT expression for all patients.

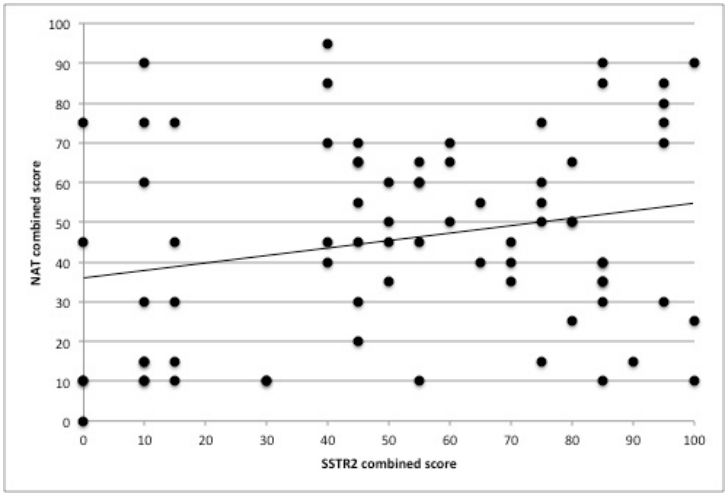


Figure 3.5B Correlation between SSTR2 and NAT for stage 4 only patients.

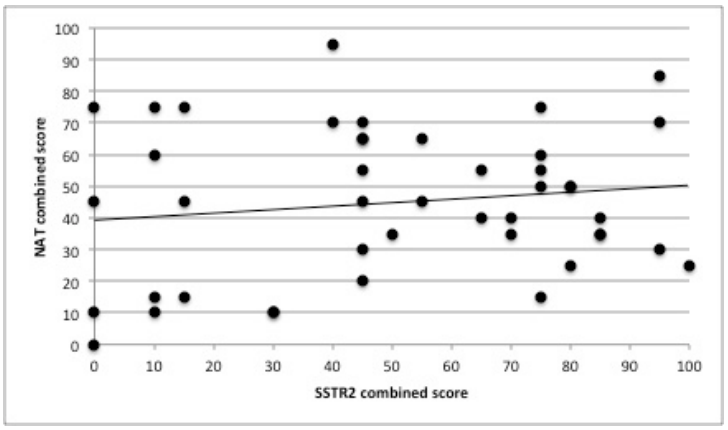


Table 3.2 A-E Baseline characteristics

Table 3.2A Gender

	M (n=44)	**F** (n=31)	**Overall** (n=75)
Combined SSTR 100			
n	44	31	75
Mean (SD)	50.9 (31.6)	57.1 (30.9)	53.5 (31.3)
Median	50	55	55
IQR	22.5, 77.5	30, 85	30, 80
Range	0, 100	10, 100	1, 100
Combined NAT 100			
n	44	31	75
Mean (SD)	44 (24.9)	48.7 (25.8)	45.9 (25.2)
Median	45	50	45
IQR	25, 65	30, 75	25, 65
Range	0, 95	10, 90	0, 95

Table 3.2B Age (months)

	< 18 months (n=35)	**> 18 months** (n=39)	**Overall** (n=74)
Combined SSTR 100			
n	35	39	74
Mean (SD)	53.3 (31.5)	54.7 (31)	54.1 (31.1)
Median	55	55	55
IQR	30, 80	30, 85	30, 80
Range	0, 100	0, 100	1, 100
Combined NAT 100			
n	35	39	74
Mean (SD)	46.9 (28.1)	44.7 (22.9)	45.7 (25.3)
Median	50	45	45
IQR	15, 65	25, 65	25, 65
Range	0, 95	10, 85	0, 95

Table 3.2C MYCN

	Yes (n=18)	**No** (n=48)	**Overall** (n=66)
Combined SSTR 100			
n	18	48	66
Mean (SD)	38.6 (26.8)	62.1 (29.4)	55.7 (30.4)
Median	40	67.5	55
IQR	15, 55	45, 85	40, 85
Range	0, 95	0, 100	1, 100
Combined NAT 100			
n	18	48	66
Mean (SD)	39.7 (22.8)	49.8 (24.6)	47 (24.4)
Median	42.5	50	45
IQR	15, 55	30, 67.5	30, 65
Range	0, 75	10, 95	0, 95

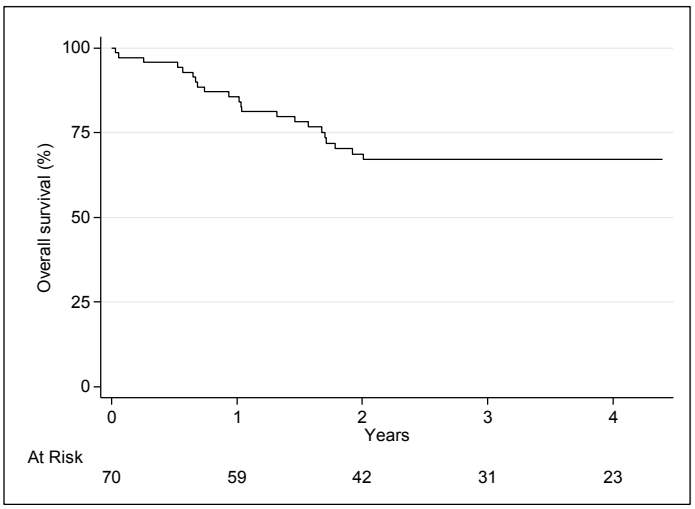
Table 3.2D Differentiated

	Differentiating (n=10)	**Poorly differentiated** (n=62)	**Undifferentiated** (n=3)	**Overall** (n=75)
Combined SSTR 100				
n	10	62	3	75
Mean (SD)	79.5 (17.6)	50.4 (30.5)	30 (43.6)	53.5 (31.3)
Median	82.5	50	10	55
IQR	60, 95	15, 75	0, 80	30, 80
Range	55, 100	0, 100	0, 80	0, 100
Combined NAT 100				
n	10	62	3	75
Mean (SD)	72 (11.6)	42.7 (24.5)	26.7 (17.6)	45.9 (25.2)
Median	70	42.5	25	45
IQR	65, 85	20, 60	10, 45	25, 65
Range	55, 90	0, 95	10, 45	0, 95

Table 3.2E Stage

	1 (n=3)	**2** (n=6)	**3** (n=6)	**4** (n=44)	**4S** (n=5)	**Overall** (n=64)
Combined SSTR 100						
n	3	6	6	44	5	64
Mean (SD)	73.3 (20.2)	46.7 (35.4)	75 (22.1)	51.6 (31.6)	28 (24.9)	52.5 (31.5)
Median	85	57.5	85	47.5	10	55
IQR	50, 85	10, 60	55, 90	22.5, 77.5	10, 50	22.5, 80
Range	50, 85	0, 95	40, 95	0, 100	10, 60	0, 100
Combined NAT 100						
n	3	6	6	44	5	64
Mean (SD)	53.3 (28.4)	53.3 (34.6)	41.7 (26.6)	44.9 (23.4)	31 (18.8)	44.7 (24.5)
Median	45	67.5	42.5	45	30	45
IQR	30, 85	10, 75	15, 60	27.5, 65	15, 50	25, 65
Range	30, 85	10, 90	10, 80	0, 95	10, 50	0, 95

Figure 3.6 Kaplan-Meir plot showing overall survival for the whole group based on 70 patients (date last seen was not available for 5 patients)



SSTR 2 Analysis

Table 3.3A The spread of patients' status for each value of combined SSTR2 score.

	Alive		Died		Overall	
Combined SSTR2 100	**n**	**%**	**n**	**%**	**n**	**%**
0	3	6.1%	2	7.7%	5	6.7%
10	5	10.2%	3	11.5%	8	10.7%
15	2	4.1%	3	11.5%	5	6.7%
30	1	2%	1	3.8%	2	2.7%
40	3	6.1%	2	7.7%	5	6.7%
45	5	10.2%	2	7.7%	7	9.3%
50	2	4.1%	2	7.7%	4	5.3%
55	3	6.1%	1	3.8%	4	5.3%
60	3	6.1%	0	0%	3	4%
65	2	4.1%	0	0%	2	2.7%
70	1	2%	2	7.7%	3	4%
75	4	8.2%	1	3.8%	5	6.7%
80	3	6.1%	1	3.8%	4	5.3%
85	5	10.2%	3	11.5%	8	10.7%
90	1	2%	0	0%	1	1.3%
95	4	8.2%	1	3.8%	5	6.7%
100	2	4.1%	2	7.7%	4	5.3%
Total	49	100	26	100	75	100

Table 3.3B SSTR2 Univariable Cox Model

The Hazard Ratio (HR) with corresponding 95% CI and p-value for the univariable Cox model for SSTR2. There is no evidence that SSTR2 has a prognostic influence on overall survival.

Variable	HR (95% CI)	p-value
Combined SSTR2 100	0.996 (0.983, 1.009)	0.558

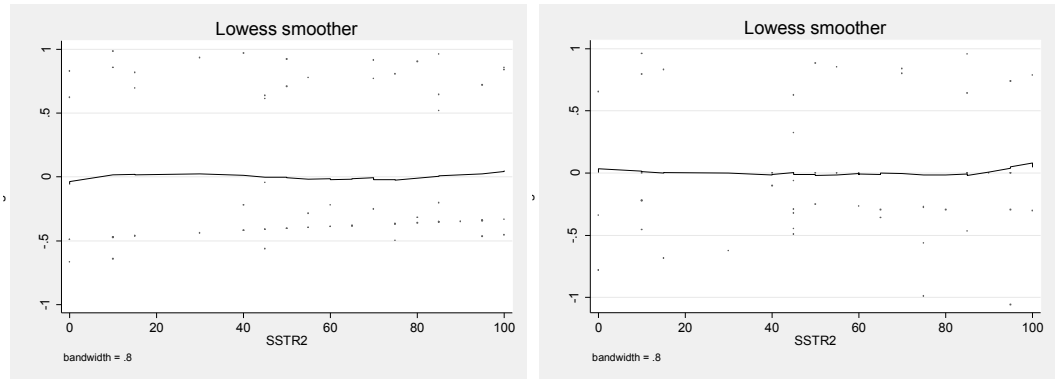
Table 3.3C SSTR2 Multivariate Cox Model

Variable	Level	HR (95% CI)	p-value
Combined SSTR2 100		1.003	0.743
Tumour stage	I, II, III IV, IVS	1.0 5.1e+16	
MYCN	Yes No	1.0 0.422 (0.142, 1.251)	 0.120
Age (years)		1.139 (0.841, 1.541)	0.4

There was no evidence to suggest that SSTR2 has prognostic influence on overall survival after adjusting for well known prognostic factors such as stage, MYCN and age.

Figure 3.7 Martingale residuals for SSTR2

The plot shows the Martingale residuals for STTR2. The plot verifies the linear assumption of STTR2 in the model (the line looks approximately horizontal).



NAT Analysis

Table 3.4A The spread of patients status for each value of combined NAT score.

	Alive		Died		Overall	
Combined NAT 100	**n**	**%**	**n**	**%**	**n**	**%**
0	0	0%	1	3.8%	1	1.3%
10	6	12.2%	4	15.4%	10	13.3%
15	3	6.1%	2	7.7%	5	6.7%
20	1	2%	0	0%	1	1.3%
25	0	0%	2	7.7%	2	2.7%
30	4	8.2%	1	3.8%	5	6.7%
35	1	2%	3	11.5%	4	5.3%
40	3	6.1%	2	7.7%	5	6.7%
45	7	14.3%	0	0%	7	9.3%
50	5	10.2%	0	0%	5	6.7%
55	1	2%	2	7.7%	3	4%
60	3	6.1%	2	7.7%	5	6.7%
65	4	8.2%	1	3.8%	5	6.7%
70	2	2%	2	7.7%	4	5.3%
75	3	6.1%	2	7.7%	5	6.7%
80	1	2%	0	0%	1	1.3%
85	1	2%	2	7.7%	3	4%
90	3	6.1%	0	0%	3	4%
95	1	2%	0	0%	1	1.3%
Total	49	100%	26	100%	75	100%

Table 3.4B NAT Univariable Cox model

The HR with corresponding 96% CI and a p-value for the univariable Cox model for combined NAT. There is no evidence to suggest that NAT has prognostic influence on overall survival.

Variable	HR (95% CI)	p-value
Combined NAT 100	0.990 (0.974, 1.007)	0.248

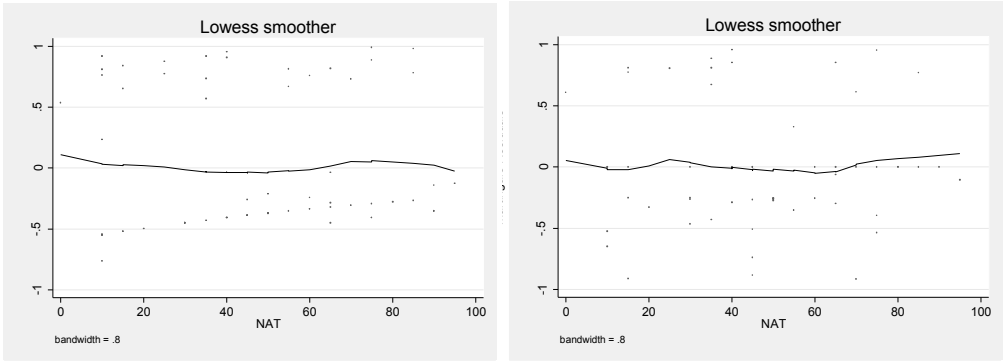
Table 3.4C NAT Multivariable Cox model

HR with corresponding 95% CI and a p-value for the multivariable Cox model for NAT. There is no evidence to suggest that NAT has prognostic influence on the overall survival adjusting for well known prognostic factors such as stage, MYCN and age.

Variable	Level	HR (95% CI)	p-value
Combined NAT 100		1.0 (0.976, 1.024)	0.989
Tumour Stage	I,II,III IV, IVS	1.0 5.0e+16	
MYCN	Yes No	1.0 0.437(0.842, 1.547)	 0.135
Age (years)		1.141 (0.842, 1.547)	0.394

Figure 3.8 NAT Martingale residuals

The plots below show the Martingale residuals for NAT. The plots verifies the linear assumption of NAT in the model (the line looks approximately horizontal) for both the invariable model (left) and the multivariable model (right).



3.4 Discussion

This study is the first to compare the protein expression of NAT and SSTR2 in a cohort of archived neuroblastoma samples. We found highly variable protein expression of NAT and SSTR2 both between patients and within individual tissue samples. There was no correlation found between the level of NAT and SSTR2. We investigated whether there was a prognostic influence of SSTR2 and NAT but found no evidence for this either alone or when corrected for other known variables such as age, stage and MYCN amplification.

The majority of the previous studies examining the expression of NAT and SSTR2 in neuroblastoma have looked at the mRNA levels rather than protein expression. There have been several *in vitro* studies that have shown that neuroblastoma cell lines that lack the expression of NAT mRNA fail to accumulate mIBG (*Carlin 2003, Lode 1995, Mairs 1994*). In other *in vitro* studies the NAT mRNA level has correlated with the extent of mIBG uptake (*Mairs 1994, Montaldo 1996, More 2011*).

A recent publication by *Dubois et al.* however, examined whether there was an association between both the tumour NAT mRNA and the NAT protein expression with mIBG avidity in patients with neuroblastoma. In contrast to the previous studies, they found no correlation between NAT mRNA expression and mIBG uptake [the median NAT expression level for 19 patients with mIBG avid tumours was 12.9% compared to 5.9% in the 8 patients with mIBG non-avid tumours (p=0.31)]. They did

however find a correlation between the NAT protein expression and mIBG avidity [the median percent expression was 50% in the mIBG avid patients compared to 10% in the mIBG non-avid patients (p=0.027)]. They also examined whether patients with mIBG avid tumours had higher NAT intensity scores. There was a trend towards this with mIBG avid tumours having higher NAT intensity scores than non-avid tumours (p=0.06). Only 1 out of the 8 mIBG non-avid tumours had a NAT protein expression >2. There were patients in the study who had low NAT protein expression and were still mIBG avid and therefore the authors have suggested that tumours with low NAT expression may accumulate mIBG via other transporters (*Dubois 2012*). There have been no studies examining whether those with greater NAT protein expression have a better response to ^{131}I-mIBG molecular radiotherapy.

There has been only one study, with 5 patients, that examined the correlation between the protein expression of SSTR2 by IHC and mRNA of SSTR2. A positive correlation was found but the comparison was only performed on 5 patients (*Raggi 2000*).

No correlation was found in this study between expression levels of NAT or SSTR2 and outcome. Previous studies have found that patients with high expression of SSTR2 mRNA had a better survival (*Sestini 1996, Brigganti 1997, Raggi 2000*). Kogner *et al.* examined somatostatin concentrations (not SSTR2 mRNA or protein expression) by radioimmunoassay in 39 children, >12 months of age and with stage 3 or 4 neuroblastoma and found that the 16 children with high somatostatin concentration had better survival than the 23 patients with low somatostatin

concentrations (*p=0.005*). In 15 children somatostatin receptor scintigraphy was performed with ^{111}In-pentetreotide. There was no significant correlation between somatostatin concentrations in tumour tissue and receptor expression as investigated by somatostatin receptor scintigraphy (*Kogner 1997*).

Orlando et al performed RT-PCR on 49 neuroblastoma samples and found a wide-range of SSTR2 mRNA expression but the lowest in stage IV patients. The patients with SSTR2 expression had a better survival. For 9 patients, they were able to find a good correlation between the concentration of SSTR2 on RT-PCR and uptake by imaging with ^{111}In-pentetreotide (*Orlando 2001*).

Several studies have shown that patients with MYCN amplification have lower expression of SSTR2 mRNA (*Sestini1996, Kogner 1997*) and *Dubois et al* reported for the first time a correlation between MYCN amplification and low NAT protein expression (*Dubois 2012*). Within our cohort of patients, the patients with MYCN amplification had lower composite scores for NAT and SSTR2 but the numbers in both groups were too small to establish whether this was significant.

As discussed above, previous studies have used varying techniques to look for SSTR2 and NAT in neuroblastomas. None of the studies have correlated SSTR2 or NAT protein expression and survival. In terms of the relevance to functional imaging and molecular radiotherapy the protein expression is more relevant than the measurement of mRNA. There have been studies in adult neuroendocrine tumours that have found patients with negative octreotide and positive mRNA (*John 1996*). As

well as studies in neuroblastoma that have found mIBG uptake in patients with low levels of NAT mRNA (*Carlin 2003*). It is also well recognized that that there can be a poor correlation between mRNA and functional protein expression possibly due to post translational modification .

For cases with multiple samples we found that the percentage of cells expressing NAT and SSTR2 increased as the tumours went from poorly differentiated to differentiated. It is unclear from the published data whether the uptake of mIBG correlates with the differentiation of neuroblastoma cells. The *in vitro* data available has supported an increase in mIBG uptake after cellular differentiation (*Montaldo 1992,Iavarone 1993*). In contrast, the first reported *in vivo* studies, supported a correlation between higher mIBG uptake in the more undifferentiated neuroblastomas (*Moyes 1989, Hadj-Djilani 1995*). However, this was not confirmed in the in vivo study by *Brans et al* who found that the more differentiated neuroblastomas in their group did not have lower mIBG uptake. They concluded that it was impossible to make judgements about the differentiation of neuroblastomas depending on their mIBG uptake (*Brans 1998*).

The uptake of mIBG is potentially influenced by many other variables such as technical factors related to the imaging technique itself and other tumour related factors for example, tumour size, tumour heterogeneity, blood supply, prior treatment and this is likely to be the same for uptake of DOTATATE by SSTR2. There are agents in clinical investigation such as Vorinostat, a HDAC inhibitor, that can increase the NAT expression (*More 2011*).

In more recent years, there have been many developments in positron emission tomography and these techniques have been shown to improve sensitivity for detection of somatostatin positive neuroendocrine tumours compared to planar scintigraphy (*Kowalski 2003, Gabriel 2007*). The gold standard would now be to correlate uptake on the latest imaging techniques such as ^{68}Ga-DOTATATE PET/CT (see chapter2) rather than octreotide planar scintigraphy and protein expression of SSTR2. ^{124}I-mIBG PET/CT is currently under investigation to establish whether it has improved sensitivity over ^{123}I-mIBG planar scinitigraphy.

This study has shown that there is a large and variable (between patients and within patients) protein expression of NAT and SSTR2 in neuroblastoma. This has implications for both functional imaging and molecular radiotherapy in the management of neuroblastoma. The variable expression of the receptors mean that not all of the tumour cells may receive the radionuclide by the chosen target. This study supports the role for both mIBG and radiolabelled somatostatin analogues for imaging and therapy to fully map and target neuroblastoma. Further more, in the future a possible combination of both molecular radiotherapy treatments targeting the two different receptors may be more effective than either used alone.

Having refined the methodology here, we will be able to apply this in the planned future clinical trials of mIBG and LuDO. Within these prospective studies, NAT and SSTR2 expression by IHC will be correlated with degree of uptake with ^{123}I-mIBG and ^{68}Ga-DOTATATE PET/CT and with response. These translational studies will help us to

understand whether those patients with higher intensity and expression of NAT or SSTR2 will have a better clinical response to their particular molecular radiotherapy.

In conclusion, there is huge variation and heterogeneity in the protein expression of NAT and SSTR2 between patients and also within the same patient. The use of immunohistochemistry could facilitate the appropriate selection of patients for molecular radiotherapy treatment.

Chapter 4

A Systematic Review of ^{131}I-*meta*-iodobenzylguanidine (mIBG) Therapy in Neuroblastoma

4.1 Introduction

Meta-iodobenzylguanidine (mIBG) is a catecholamine derivative which has a high specificity for the Noradrenaline transporter molecule (NAT). Approximately 90% of neuroblastomas express the noradrenaline transporter molecule (NAT) and mIBG can be radiolabelled with either ^{123}I for imaging, or ^{131}I for therapy.

mIBG was developed at the University of Michigan in the mid 1980's and the first tumours to be imaged and then treated with mIBG were phaeochromocytomas *(Sisson 1981, Sisson 1983, Vetter 1983*).

In 1987, just four years after the first reports of ^{131}I-mIBG therapy in pheochromocytoma were published, nine groups presented results of early clinical experience with this agent in neuroblastoma (*Beierwaltes 1987, Bestagno 1987, Cottino 1987, Fischer 1987, Hartmann 1987, Sanguinetti 1987, Treuner 1987, Troncome 1987, Voûte 1987*). As is often the case with the introduction of new cancer treatments, early clinical evaluation was performed on end-stage neuroblastoma patients, for whom there was no conventional treatment or established benefit available. Among these patients, there were four patients with a complete response, 28 with a partial response, and 12 patients without disease progression. These results need to be viewed with a degree of caution, as the patient population was heterogeneous, the administration of ^{131}I-mIBG therapy was not standardized, and the criteria to judge a response were not uniform.

Nonetheless, these early results indicated that ^{131}I-mIBG was a promising treatment for neuroblastoma, and worthy of further evaluation.

The dose-limiting toxicity of ^{131}I-mIBG is myelosupression. Haemopoietic support, typically with peripheral blood stem cells (PBSC), has been used to circumvent myelosuppression, and to safely facilitate the use of higher Administered Activities (AAs). Other attempts to improve outcome include the use of concomitant chemotherapy or radiosensitisers, hyperbaric oxygen and dosimetry-guided administrations. ^{131}I-mIBG therapy, originally used for refractory or relapsed neuroblastoma, has also been incorporated into induction and consolidation treatments.

Despite nearly 30 years of use of ^{131}I-mIBG molecular radiotherapy in neuroblastoma, the effectiveness of ^{131}I-mIBG therapy remains uncertain, and its optimal use remains undefined. A systematic review of ^{131}I-mIBG therapy in neuroblastoma has therefore been undertaken with the aim of improving the understanding of the published data and defining uncertainties to be addressed in future clinical trials.

This work was undertaken in collaboration with Jayne Wilson who is a systematic reviewer at the Cancer Research UK, Clinical Trials Unit, University of Birmingham. The systematic review has been published in the European Journal of Cancer (*Wilson 2013*).

4.2 Materials and Methods

Standard systematic review methods were employed following a pre-defined protocol. We searched MEDLINE, EMBASE, and Cochrane CENTRAL bibliographic databases from inception to July 2012 using terms for neuroblastoma and ^{131}I-mIBG.

The Inclusion criteria were:

- Population/setting – patients with neuroblastoma of any age and no matter what prior therapy they had received (including chemotherapy, surgery, radiotherapy or other treatments)
- Intervention – ^{131}I-mIBG therapy (distinct from diagnostic mIBG scans) alone or in combination with other treatments given at any stage of disease trajectory (induction, refractory, consolidation or relapse) for both curative and palliative intent
- Comparator – any or none
- Design - systematic reviews, Phase I, Phase II, and Phase III trials, prospective and retrospective non-comparative case series with 10 or more patients;
- Outcomes – at least one of tumour response, overall survival (OS), event free survival (EFS), toxicity, adverse events, quality of life, symptom control and dosimetry.

All references were reviewed by the two reviewers (JG and JW) and independently assessed for inclusion/ exclusion criteria using the title and abstract. Disagreements about inclusion/exclusion of papers were resolved by discussion. Full copies of the potentially relevant papers were then obtained for detailed examination.

Study characteristics and results were extracted using a pre-designed data extraction form by one reviewer and checked by a second reviewer with discrepancies discussed where identified.

Mean cumulative administered activity (AA) per patient per study was calculated by summing the total AA per patient and dividing by the number of patients who were prescribed or received ^{131}I-mIBG. Six studies reported AA/kg of body weight, meaning it was not possible to calculate cumulative AA. However, these studies gave individual patient results (resulting in 18 data plots) according to AA/kg, therefore we were able to test for AA against response.

Data from studies with multiple publications were extracted and reported as a single study, in case of data discrepancies the most recent data was utilized. Study quality was assessed using an adaption of the tool from York CRD (*Wilson 2005*) with the aim to identify selection, detection and attrition bias.

Studies were assigned to one of three groups. Induction studies included studies where ^{131}I-mIBG therapy was used as the first line treatment, up-front in newly diagnosed patients. Consolidation studies included those where ^{131}I-mIBG was given

in conjunction with myeolablative chemotherapy after a response had been seen with induction chemotherapy. Relapsed/refractory studies included those where ^{131}I-mIBG therapy was used in patients whose disease had progressed after an initial response to therapy or those who had not responded to induction therapy. Relapsed and refractory patients are likely to be different and respond differently and ideally would be reported separately but most papers put the two groups together and did not report the outcomes separately.

Objective tumour response was defined using the International Neuroblastoma Response Criteria as complete response (CR), or partial response (PR) (*Brodeur 1993, Brodeur 1998*). Mixed response (MR), stable disease (SD) and no response (NR) did not contribute to the objective response outcome.

Statistical methods

For each study, the proportion of patients with objective tumour response with 95% confidence interval (CI) was presented. Odds ratios (OR) with CIs were derived using logistic regression for grouped data with robust standard errors. Two covariates were included in the model: AA (numerical) and concurrent chemotherapy administration. Odds ratios greater than 1.0 indicated an increased likelihood of response with increasing AA or with chemotherapy. Stata v12 was used for the calculations.

4.3 Results

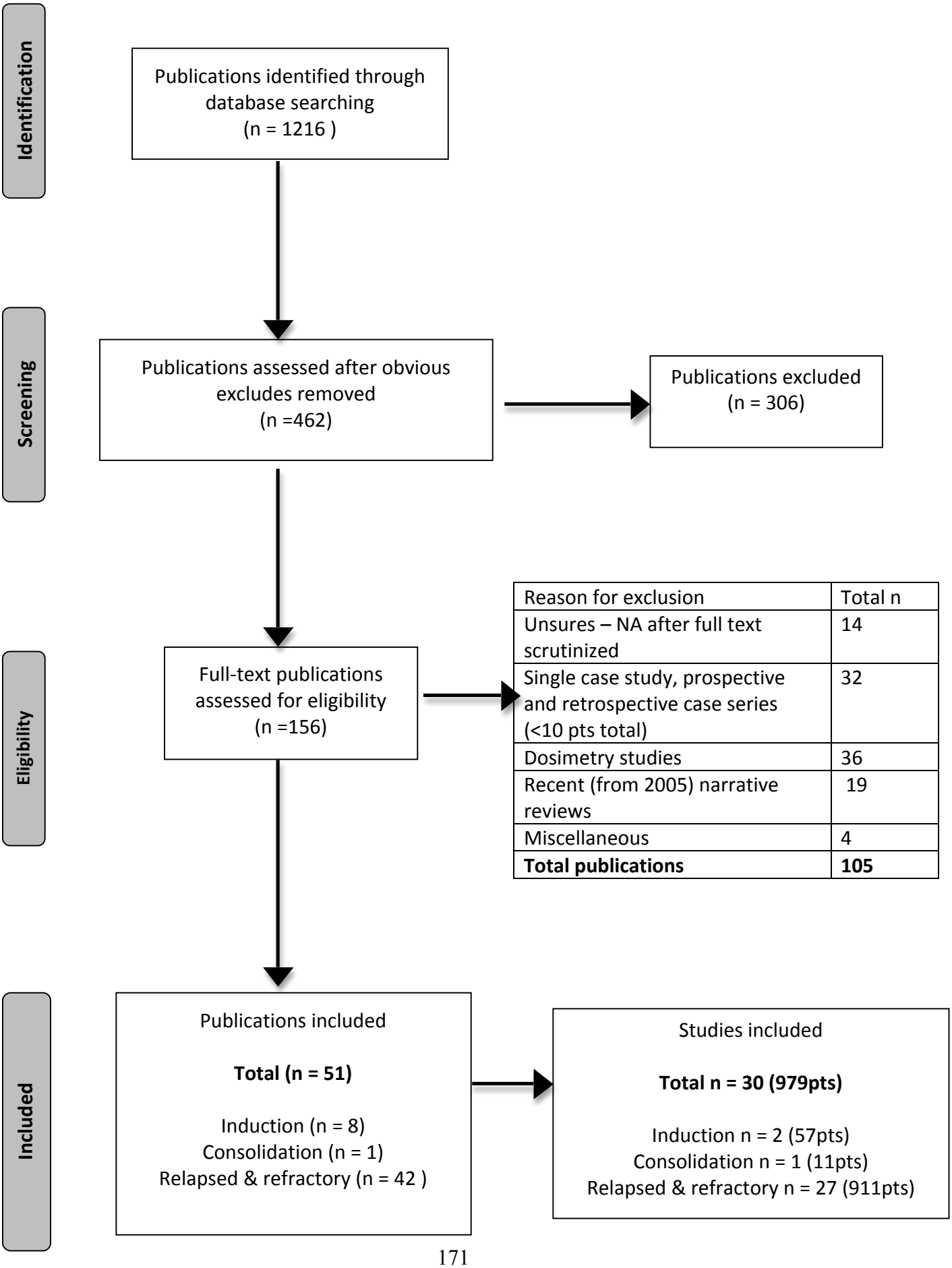
Overall Quantity of Literature

The electronic searches yielded 1,216 citations of which 51 publications reporting 30 studies met the inclusion criteria (see Figure 4.1 PRISMA diagram).

Of these, two studies examined the role of ^{131}I-mIBG as an induction therapy, one study as consolidation therapy and 27 studies as a treatment option in relapsed and refractory patients. Twenty-two were prospective case series, four were retrospective case series, and four studies had non-randomly allocated control groups. No randomized controlled trials (RCTs) were identified in the search.

The main reasons for exclusion were because the study focus was purely dosimetry based (n=36), or because the studies were either single case studies or prospective /retrospective case series with less than 10 patients (n=32).

Figure 4.1 – PRISMA flow diagram



Reason for exclusion	Total n
Unsures – NA after full text scrutinized	14
Single case study, prospective and retrospective case series (<10 pts total)	32
Dosimetry studies	36
Recent (from 2005) narrative reviews	19
Miscellaneous	4
Total publications	**105**

Induction Studies

Eight papers reporting two studies involving 57 patients where ^{131}I-mIBG was used in the induction setting were identified (see Table 4.1) (*De Kraker 2008, Mastrangelo 2011*).

Patients within these studies had INSS stages 3 and 4 disease with a mean age of 2.6 years. ^{131}I-mIBG was given alone (median 3 administrations – range 2 to 5) followed by surgery (*De Kraker 2008*) or as a single administration with concomitant chemotherapy (*Mastrangelo 2011*). Mean cumulative AA was 11.1GBq (De Kraker 2008) and 6.26GBq (*Mastrangelo 2011).*

Study quality was good for de Kraker (2008) but there may have been some selection bias in Mastrangelo (2011) (see table 4.4). Inconsistent reporting between the large number of multiple publications was problematic.

Overall between the two studies objective tumour response was measured in 39/57 (68%) patients, the majority classed as a PR (see table 4.5). For de Kraker the objective tumour response was measured in 27/44 (61%) patients and for Mastrangelo 12/13 (92%) patients.

In De Kraker (2008) median OS was reported as 15 months (CI 7 to 23), with 5 year OS at 15%. Mastrangelo (2011) reported 3 deaths, with four patients alive with CR

(range of duration 1.5 to 8.4 years) and one patient alive with SD. Median OS was not reported, median EFS was 10 months (CI 7 to 13 months) and 5 year EFS was 12%.

De Kraker et al. also evaluated surgical success following ^{131}I-mIBG induction. Out of 24 patients who received ^{131}I-mIBG alone, 18 (75%) had tumours surgically resected. In 17 patients, there was an initial poor response to ^{131}I-mIBG and so chemotherapy was also administered. Of these 17 patients, 8 (45%) went on to have their tumours surgically resected. Overall, 9 patients did not receive surgery because of progressive disease, 2 because of multiple inoperable tumors and 2 did not have a primary tumour. One patient died form surgical complications (*De Kraker 2008).*

Table 4.1 Induction Studies Interventions

Study	Total no. patients	Intervention	^{131}I-mIBG AA per cycle (range)	No of ^{131}I-mIBG cycles (range)	Mean cumulative AA (GBq)	Chemo-therapy	Stem cell support (PBSC)
De Kraker 2008 Hoefnagel 1995 De Kraker 1995 Hoefnagel 1994 Hoefnagel 1991 Van Hasselt 1996	44	^{131}I-mIBG therapy alone followed by re-assessment after 2 cycles. Group A responding – further ^{131}I-mIBG before surgery. Group B SD or PD – chemotherapy.	Fixed AA: 7.4GBq. Subsequent cycles 3.7GBq.	Median 3 (2-5)	N/A	Yes for 17 pts	No
Mastrangelo 2011 Mastrangelo 2001	13	^{131}I-mIBG combined with chemotherapy. ^{131}I-mIBG given on day 10.	Fixed AA 7.4GBq. (Younger pts 5.55GBq)	1	N/A	Yes	No

Consolidation Studies

Only one study met the inclusion criteria for a consolidation study (*Klingebiel 1998).* This study recruited 11 patients, all with INSS stage 4 disease after initial chemotherapy on the German Neuroblastoma Study BN90 and were given the option to enrol for ^{131}I-mIBG treatment. The mean age was 5 years. Patients received a single cycle of ^{131}I-mIBG, AA 0.58GBq/kg. This was immediately followed by high-dose chemotherapy, PBSC support and then antiGD2 antibody (see Table 4.2).

It is noted that those studies which used intensive or myeloablative schedules for the treatment of patients who were not in remission because they had had no response, or only a poor response, to induction treatment are reported as studies in refractory and relapsed disease, not as consolidation studies.

Table 4.2 Consolidation Study Interventions

Study	Total no. patients	Intervention	^{131}I-mIBG AA per cycle (range)	No of ^{131}I-mIBG cycles (range)	Mean cumulative AA GBq	Chemo-therapy	Stem cell support (PBSC)
Klingebiel 1998	11	4-8 cycles of induction chemotherapy on NB90 trial. ^{131}I-mIBG consolidation followed by high-dose chemotherapy, PBSC and antiGD2 antibody.	WB AA 0.58GBq/kg	1	N/A	Yes	Yes

Objective tumour responses, assessed after recovery from stem cell transplantation, were identified in 4/11 patients and 4/11 patients had a continued response from induction. Two had PD and one suffered a relapse. OS was 70% at 19 months, EFS was not reported.

Relapsed and Refractory Studies

Twenty seven studies met the inclusion criteria. By 'refractory patients' we meant those patients whose disease had responded poorly or not at all to initial (induction) chemotherapy, and who had not proceeded to consolidation with myeloablative therapy. By 'relapsed patients' we meant those whose disease had progressed after an initial partial or complete response, and often after consolidation with myeloablative therapy. However, in various publications there was not always a clear distinction between refractory and relapsed disease.

Eight of the relapsed and refractory studies had multiple publications (Hoefnagel 1987, Treuner 1988, Lumbroso 1991, Hutchinson 1992, Lashford 1992, Garaventa 1999, Dubois 2004, Matthay 2007). There were a total of 42 publications (see Table 4.3). Including the control patients, the total number of patients was 1,121. The study size ranged from 10 to 164 patients. Publication dates spanned from 1987 to 2012. One study each came from the UK, France and Spain, two studies came from The Netherlands, six from Germany and Italy and 10 from the USA.

Sixteen studies gave recruitment dates, the earliest to start was 1984 (*Treuner 1988, Klingebiel 1991a and 1991b*) and the latest 2005 (*Matthay 2007, Matthay 2009*). The mean recruitment duration was 5 years but ranged form 1 (*Treuner 1988*) to 10 years (*DuBois 2004*).

The lowest mean age range in a study was 1.9 years (*Castel 2000*), the highest was 8 years (*Castellani 1991, Matthay 2012*). Median mean age across the studies was 4.5 years, with a wide range of individual ages from 0.3 years to 36 years. Thirteen studies reported age at diagnosis, two reported age at both diagnosis and start of treatment, five reported age at start of treatment and in eight it was unclear.

Three different neuroblastoma staging systems for diagnosis had been utilized (*Evans 1971*, *Brodeur 1988, Brodeur 1993*). Eleven studies used Evans 1971, two used INSS 1988, seven used INSS 1993 and 8 studies did not give stage information.

Two patients had stage 1 disease (*Dubois 2004, Johnson 2011*), three patients had stage 2 disease (*Castel 2000, Johnson 2011, Voûte 1995*) and 2 patients had stage 4S disease (*Dubois 2004, Miano 2001*). The majority of patients had either stage 3 (*n* = 192 patients) or stage 4 disease (*n* = 403 patients). The proportion of patients with MYCN amplification was reported in only five studies: 39% (*Dubois 2004*); 33% (*Yanik 2002*); 25% (*Matthay 2006*); 12% (*Castel 2000*), 15% (*Schmidt 2006*).

The most frequent disease sites were the abdomen, bone and bone marrow. However, inconsistent reporting gives an unreliable estimate as to the total extent of the disease burden.

Where it was reported, the vast majority of patients had had extensive prior treatments. This included a range of multi-drug induction regimens in refractory patients, and a greater number of second and third line chemotherapy schedules in relapsed patients. Around a quarter of the patients were reported to have had high-dose (HD) chemotherapy and PBSC, and a third had had prior surgery, with a small proportion having had external radiotherapy. Again, inconsistent reporting may mean that the true figures are higher and cannot give us an accurate assessment of prior treatment.

^{131}I-mIBG was given alone in 20 studies and with concurrent chemotherapy in seven. For the ^{131}I-mIBG alone studies the number of cycles ranged from 1 to 6, whereas ^{131}I-mIBG given with concurrent chemotherapy tended to have fewer cycles ranging from 1 to 3. A wide range of AA were given [Table 4.3]. Authors reported planned and/or observed AA [Table 4.6].

Table 4.3 Relapsed and refractory study interventions

Study	Total no. patients (Relapsed/ refractory/not stated)	Intervention	^{131}I-mIBG AA per cycle (range)	No of ^{131}I-mIBG cycles (range)	Mean cumulative AA GBq	Chemo-therapy	Stem cell support (PBSC)
Castel 2000	35 (10/25)	Continuous infusion chemotherapy combined with ^{131}I-mIBG	Fixed AA: 7.4GBq	3	7.39	Yes	No
Castellani 2000	Group 1:14 (14/0) Group 2:8 (0/8)	^{131}I-mIBG alone every 4 weeks	Fixed AA Group 1: 14.39GBq Group 2: 9.05GBq	1 to 6 for advanced disease. 1 to 2 for residual disease after primary treatment	Group 1 10.31. Group 2 7.82.	No	No
Claudiani 1991	42 (NS)	^{131}I-mIBG alone	WB AA <15kg(2.5-3.7GBq) 15-20kg(3.7-4.7GBq) >20kg(4.7-5.5GBq)	1=15 pts 2=6pts 3=18pts 4=3pts 6=1pt	NC	No	No
Dubois 2012	24 (18/6)	^{131}I-mIBg on day 1 with chemo.	WB AA 0.296GBq/kg, 0.444GBq/kg, 0.555GBq/kg, 0.666GBq/kg	1=17 pts 2=5 pts 3= 2 pts	AA/Kg	Yes	Yes
Dubois 2004 Matthay 1991 Matthay 2001 Goldberg 1998 Polishchuk 2011	53 (53/0)	^{131}I-mIBG alone. Single administration.	WB AA 0.666GBq/kg	1	11.47 reported in Matthay 2001	No	Yes
Garaventa 1999 Garaventa 1995	43 (0/43)	^{131}I-mIBG alone	WB AA <15kg(2.5-3.7GBq) 15-20kg(3.7-4.7GBq) >20kg(4.7-5.5GBq)	Median 3 (1-5)	9.6	No	No

Study	Total no. patients (Relapsed/ refractory/not stated)	Intervention	^{131}I-mIBG AA per cycle (range)	No of ^{131}I-mIBG cycles (range)	Mean cumulative AA GBq	Chemo-therapy	Stem cell support (PBSC)
Hoefnagel 1987 Voute 1991	16 (1/15)	^{131}I-mIBG alone	AA 1.5-7.4GBq	1	10.78	No	No
Hutchinson 1992 Hutchinson 1991 Bierwaltes 1987	14 (14/0) (11 additional pts were case matched controls)	^{131}I-mIBG alone 12 to 16 week intervals	Fixed AA Dose escalated Median 5.7GBq (1.9-8.1Gbq)	1-3	9.84	No	No
Johnson 2011	76 (42/13/NS 21)	^{131}I-mIBG alone (tandem infusions)	WB AA 0.666GBq/kg	1=23pts 2=53pts	AA/Kg	No	No
Kang 2003	17 (NS)	^{131}I-mIBG alone	1st administration 0.3GBq/kg (0.1-0.5GBq/kg) 2nd 9.1(5.8-13.2GBq) 3rd 4.3(9-12GBq)	1=17pts 2=8pts 3=4ts	NC	No	No
Klingebiel 1991a	47 (16/31) 30 pts in control group – pt characteristics NS	^{131}I-mIBG alone	Observed mean dose/kg/course 0.3293GBq +/- 0.2701GBq	Stage III mean 2 (1-5) Stage IV mean 2.5(1-6)	Stage III 10.48. Stage IV 14.39.	No	No
Klingebiel 1991b	25 Group A=14 (0/14) (tox/response assessment) Group B= 8(0/8) (multimodal assessment also had BMT and immunotherapy)(Stage III 0/3)	^{131}I-mIBG alone (stage III and Iv Group A), single or double autologous stem cell transplant and immunotherapy (Stage IV Group B) ◆	Planned dose: Stage III: median 0.322GBq/kg Stage IV Group A: median 0.296GBq/kg Stage IV group B: median 0.74GBq/kg	Stage III median 1 cycle (1-2) Stage IV: Group A: median 2 (1-5) Group B all 1 cycle	10.39	Yes	Yes
Lashford 1992 Lewis 1991	25 (0/25)	^{131}I-mIBG alone	AA prescribed to WB dose from tracer study: range 2.4-12.1 GBq to achieve WB dose of 1-2.5Gy	1	6.38	No	No
Lumbroso 1991	26(14/12)	^{131}I-mIBG alone in 1 monthly intervals	Observed dose median 2.6GBq(1.1-4GBq)	1=14 pts 2=8 pt 3=1 pt 4=2 pt 5=1 pt	5	No	No

Study	Total no. patients (Relapsed/ refractory/not stated)	Intervention	^{131}I-mIBG AA per cycle (range)	No of ^{131}I-mIBG cycles (range)	Mean cumulative AA GBq	Chemo-therapy	Stem cell support (PBSC)
Mastrangelo 2011 Mastrangelo 2001	16 (16/0)	^{131}I-mIBG in combination with multi-drug chemo. ^{131}I-mIBG given on day 10.	Fixed AA 7.4GBq	1	6.26	Yes	No
Matthay 2006	24 (8/12/NS 3)	^{131}I-mIBG in combination with high-dose chemo and ASCT	Phase I 3+3 design. Dose escalation from (0.444GBq/Kg) to (0.666GBq/Kg). MTD was (0.444GBq/Kg) in combination with CEM.	1	AA/Kg	Yes	Yes
Matthay 2007 Howard 2005	164 (138/26)	^{131}I-mIBG alone	Planned dose 0.666GBq/Kg if stem cells available, 0.444GBq/Kg if no stem cells.	35 patients had multiple cycles (up to 4)	AA/Kg	No	Yes
Matthay 2009	21 (16/5)	^{131}I-mIBG and ASCT	Planned Phase I 3+3 dose escalation: 0.444GBq/Kg to 0.777GBq/Kg	1	AA/Kg	No	Yes
Matthay 2012	15 (13/2)	No carrier added ^{131}I-mIBG alone	3+3 dose escalation: 0.444GBq/Kg to 0.777GBq/Kg	1	AA/Kg	No	Yes
Miano 2001	Intervention 17 (0/17) Control (NS 15)	^{131}I-mIBG followed by HD chemo and PBSC rescue	Observed median 0.259GBq/Kg (0.152-0.411GBq/Kg)	1	NC	Yes	Yes
Schmidt 2006	Intervention 40 (0/40) Control (0/71)	^{131}I-mIBG randomized to receive either before mega therapy or after maintenance therapy	AA median: 0.44GBq/Kg (0.14-1.46GBq/Kg)	1	NC	Yes	Yes
Schwabe 1987 Hor 1991	11 (NS)	^{131}I-mIBG alone	Planned dose, activity divided into 2 single doses 24 hr apart: 1st administration 0.37GBq/Kg, 2nd administration given if WB dose <2Gy and liver <5Gy	1-6	14.69	No	No

Study	Total no. patients (Relapsed/ refractory/not stated)	Intervention	^{131}I-mIBG AA per cycle (range)	No of ^{131}I-mIBG cycles (range)	Mean cumulative AA GBq	Chemo-therapy	Stem cell support (PBSC)
Treuner 1987 Treuner 1986	10 (NS)	^{131}I-mIBG alone	Observed mean AA: 6GBq (1.9-13.5GBq)	Mean 2.5	NC	No	No
Treuner 1988	27(12/15)	^{131}I-mIBG alone	Observed dose mean: 5GBq (1.3-16.6GBq)	Mean 2.6 (1-6)	13.21	No	No
Troncone 1991	11 (1/10)	^{131}I-mIBG alone 1-2 monthly intervals	Observed fixed AA (2.6GBq to 9.5GBq)	Up to 4	13.64	No	NO
Voute 1995	^{131}I-mIBG alone=36 (36/0) ^{131}I-mIBG + HBO= 27 (27/0)	^{131}I-mIBg alone or with HBO. 1-2 monthly intervals	Fixed AA: 1st administration 7.4GBq, subsequent 3.7GBq	Up to 4	N/A	No	No
Yanik 2002	12 (12/0)	^{131}I-mIBG combined with myeloablative chemo and ASCT	WB AA: 0.444GBq/Kg	1	7.84	Yes	Yes

Study Quality

The quality of the studies was highly variable (see Table 4.4). Overall, the reporting of recruitment processes and patient description at study entry appears to be highly variable across and within studies, meaning that most of the studies would be subject to a degree of selection bias.

There also appears to be a high possibility of detection bias, although there is less variation within the studies than between studies, meaning that some studies were relatively unbiased and some heavily biased.

Six scales for assessing tumour response were used over the studies (see Table 4.6) and there was variability as to at what time point response was measured. There was a low probability of attrition bias with most of the studies accounting for all of the patients recruited. The main problem across the studies was inconsistency of reporting particularly amongst the studies with multiple publications.

Table 4.4 Study quality assessment. A matrix showing objective assessment of the possibility of bias in each study.

Study ID	de Kraker J 2008	Mastrangelo S 2011	Klingebiel T 1998	Castel V 2000238	Castellani MR 2000	Claudiani F 1991	Dubois SG 2004	Dubois SG 2012	Garaventa A 1999	Hoefnagel CA 1987	Hutchinson RJ 1992	Johnson K 2011	Kang TI 2003	Klingebiel T 1991	Klingebiel T 1991	Lashford LS 1992	Lumbroso J 1991	Mastrangelo S 2001	Matthay KK 2006	Matthay KK 2007	Matthay KK 2009	Matthay KK 2012	Miano M 2001	Schmidt M 2005	Schwabe D 1987	Treuner J 1987	Treuner J 1988	Troncone l 1991	Voûte PA 1995	Yanik GA 2002
Recruit. methods described?	+	-	?	-	-	-	-	-	+	-	+	-	+	+	+	-	-	?	?	-	?	?	-	+	+	-	-	-	?	-
Place of recruit. stated?	-	+	?	+	+	+	+	-	+	?	+	+	+	-	-	+	?	+	+	+	+	-	-	+	+	-	-	+	+	+
Explicit inclusion criteria?	+	-	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-	-	+	+	+	+	-	+	-	-	-	-	-	+
Entry at similar point in disease?	+	-	+	-	+	+	-	?	+	?	-	?	+	+	-	+	+	+	-	-	+	+	+	+	+	+	?	+	+	-
Long enough follow-up?	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Outcome defined?	+	+	-	-	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+
Blinding response assessor?	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	N / A	-	-	-	-	N / A	-
Time of assess. specified?	+	+	-	+	-	+	+	+	+	-	-	?	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+
All pts. account. for at end of study?	+	+	+	-	+	+	-	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+

Efficacy Results

All but two studies (*Voûte 1995, Schmidt 2006*) measured tumour response [Table 4.5]. Overall mean tumour response was 32% (253/782), but as can be seen in Figure 4.2 there are a wide range of proportions for each individual study. For refractory patients, the overall tumour response was 37% (61/164), for relapsed patients it was 38% (43/113), and in the studies with mixed populations, where response was not reported separately for relapsed and refractory patients, it was 30% (149/505).

In the multivariable analysis of cumulative AA, and chemotherapy (used or not), there is a positive association between response rate and cumulative AA (OR 1.15; CI 1.06 to 1.24; p = 0.001) and for chemotherapy (1.80; CI 1.04 to 3.15; p = 0.035). Similar results were obtained when the studies with chemotherapy are omitted (univariate analysis OR 1.17 : CI 1.07-1.27; p<0.001). However, there was no clear evidence of a relationship between response and AA/kg (OR 0.52; CI 0.21 to 1.30; p = 0.16) nor chemotherapy with OR 0.66 (CI 0.32 to 1.36; p = 0.26), with the point estimates being suggestive of a possible negative association. Again omitting the chemotherapy studies and undertaking a univariate analysis gives similar values (OR 0.54; CI 0.22-1.34 p=0.183).

Median OS was reported (Dubois 2004, Huthinson 1992, Matthay 2007, Klingebiel 1991, Matthay 2009, Johnson 2011, Matthay 2006) in seven studies. This ranged from 6 months (Huthinson 1992) to 48 months (Matthay 2006). OS at 1 year varied

from a low 38% to 100% and was reported in eight studies (Huthinson 1992, Matthay 2007, Treuner 1988, *Klingebiel 1991b, Matthay 2009, Castel 2000, Johnson 2011, Matthay 2006*).

Table 4.5 Tumour response results. The response rates and the scales used to assess response are shown.

Study	Rate	Total Study No.	No. OR	CR	PR	PD	SD/NR/ MIX/ Min/NE	NE
	Induction							
*de Kaker J 2008	61%	44	**27**	1	26	4	9	1
Mastrangelo S 2011	92%	13	**12**	2	10	0	1	0
Total	68%	57	**39**	3	36	4	8	3
	Relapsed and Refractory							
^{131}I-mIBG plus Chemotherapy								
Castel V 2000	17%	35	**6**	NS	NS	6	3	
Dubois SG 2012	25%	24	**6**	NS	NS			
Klingebiel T 1991	32%	25	**8**	2	6		4	2
Mastrangelo S 2001	75%	16	**12**		12		4	
Matthay KK 2006	25%	24	**6**	1	5	1	15	
Miano M 2001	47%	17	**8**	5	3		9	
Yanik GA 2002	67%	12	**8**	6	2	1	3	
Total	39%	124	48	14	28	5	35	2
^{131}I-mIBG alone								
Castellani MR 2000 Group	14%	14	**2**		2		9	
Castellani MR 2000 Group	38%	8	**3**		3	1	4	
Claudiani F 1991	17%	42	**7**	2	5	12	23	
Garaventa A 1999	30%	43	**13**	1	12	1	24	
Hoefnagel 1987	44%	16	**7**	2	5		5	
Hutchinson RJ 1992	7%	14	**1**		1		3	
Johnson K 2011	30%	76	**23**	5	18	16	37	
Kang TI 2003	24%	17	**4**		4		10	3
Klingebiel T 1991 Stage III	45%	11	**5**	1	4		6	
Klingebiel T 1991 Stage IV	47%	36	**17**	8	9		19	
Lashford LS 1992	32%	25	**8**		8	7	9	1
Lumbroso J 1991	4%	26	**1**		1	7	6	10
Matthay KK 2001 (part of Dubois SG2004 53pts)	38%	42	**16**		16	9	17	
Matthay KK 2007	36%	164	**59**	13	46	44	60	
Matthay KK 2009	10%	21	**2**		2	7	12	
Matthay KK 2012	27%	15	**4**	1	3	4	7	
Schwabe D 1987	64%	11	**7**	1	6		1	3
Treuner J 1987	40%	10	**4**	2	2	1	5	
Treuner J 1988	52%	27	**14**	4	10	5	5	
Troncone L 1991	18%	11	**2**	1	1	2	5	
Total	32%	629	199	41	158	116	267	17
Total relapsed and refractory	**32%**	**782**	**253**	**55**	**186**	**124**	**305**	**19**

TR = tumour response, OR = overall response (CR + VGPR + PR), CR = complete response, PR = partial response, SD = stable disease, NR = no response, PD = progressive disease, MIX = mixed response, Min = minimal response, NE = not evaluated. NS = response not stated, therefore totals for CR and PR missing 12 patients. Not all patients accounted for therefore totals will not add up. NB individual % rounded up.
*After MIBG x 2.

Table 4.6 Response Scales used

Scale used	Compatibility with INCR?
International Neuroblastoma Response Criteria (INRC)	Yes (Dubois 2004, Garaventa 1999, Matthay 2007, Matthay 2009, Yanik 2002, Matthay 2006, Mastrangelo 2001, Kang 2003
European Neuroblastoma Study Group (ENSG)	No (Lashford 1992)
International Union Against Cancer	PR similar to definition in INCR (Castellani 1991)
New Approaches to Neuroblastoma Therapy (NANT)	Yes, modified INCR (Matthay 2012, Dubois 2012)
Modified INCR	Yes (Johnson 2011)
Not stated	Unknown (Treuner 1988, Kilngebiel 1991a and b, Castel 2000, Miano 2001, Treuner 1987) PR definition given and similar to INCR (Hoefnagel 1987)

Table 4.7 Planned or observed doses

	Planned study	Observed study	Total
Cumulative AA	(n = 5) (Hutchinson 1992, Klingebiel 1991, Yanik 2002, Mastrnagelo 2001, Schwabe 1987)	(n = 8) (Garaventa 1999, Lashford 1992, Lumbroso 1991, Hutchinson 1992, Klingebiel 1991, Castel 2000, Castellani 1991, Troncone 1991)	12 studies
AA/kg	(n = 6) (Matthay 2006, 2007, 2009, 2012, Johnson 2011, Dubois 2012)		6 studies
Not included in graphs as mean dose not calculable	(n = 3) (Dubois 2004, Hoefnagel 1987, Claudiani 1991)	(n = 4) (Treuner 1986, 1988, Miano 2001, Kang 2003)	7 studies
No tumour response data presented	(n=1) (Voute 1995)	(n = 1) (Schmidt 2006)	2 studies

Some trials report planned doses i.e. the dose the patient was intended to receive, whereas some studies reported observed doses i.e. the actual dose received. Only one study[15] reported both.

Figure 4.2 Overall Objective Tumour Response for relapsed and refractory, consolidation and induction studies shown as reported response rates.

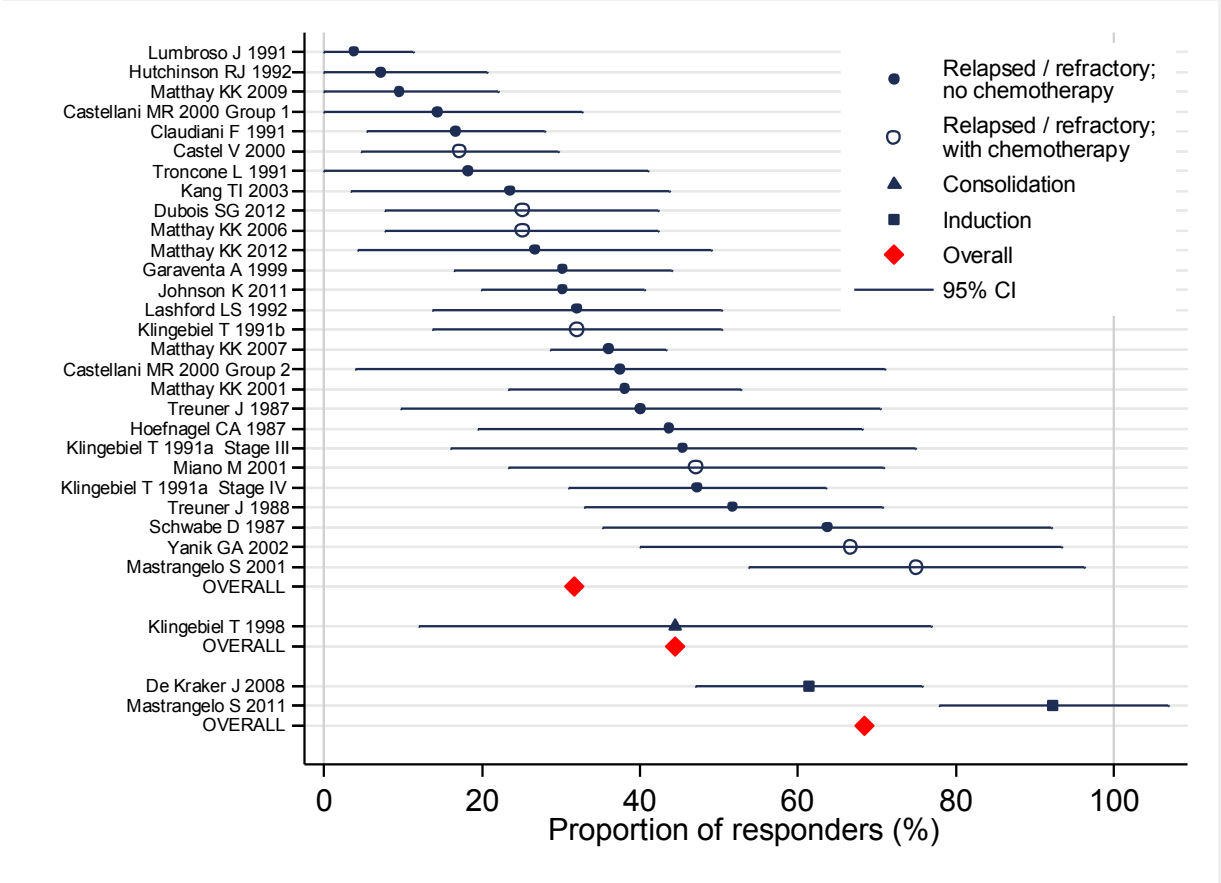
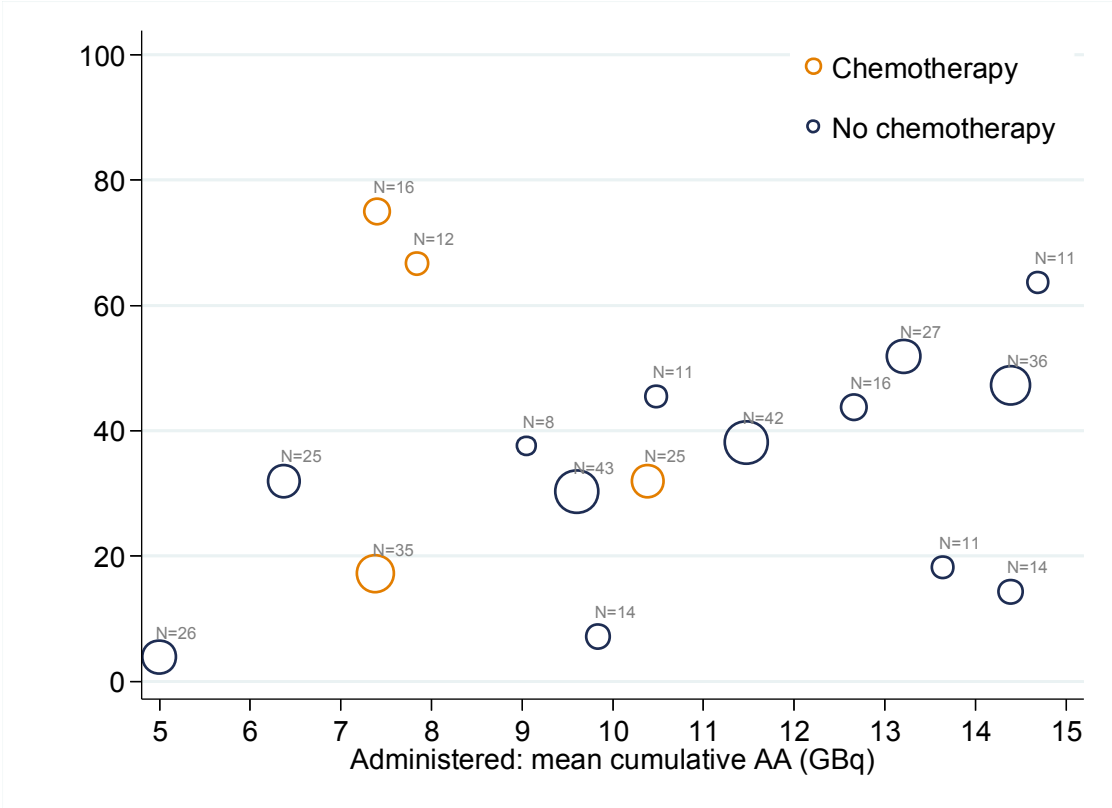


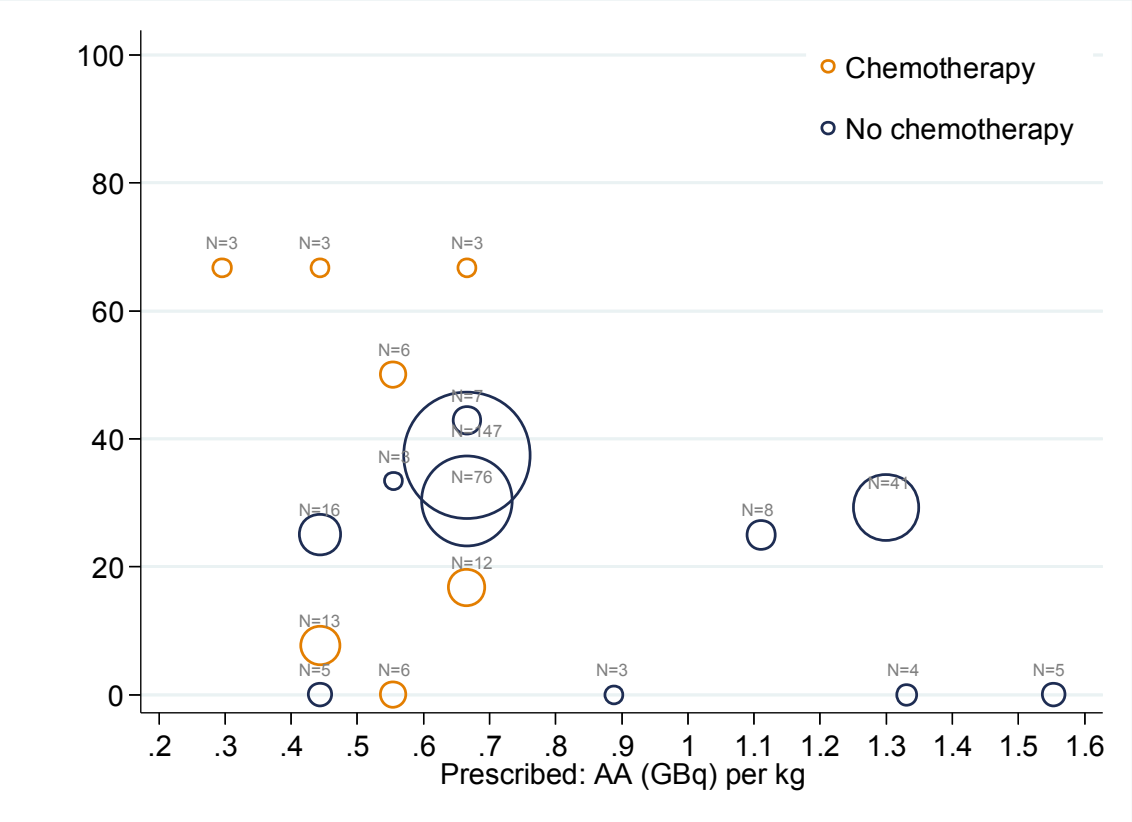
Figure 4.3 shows the scatter plot of proportion of responders against mean cumulative administered activity (AA) to assess whether a dose response relationship existed and whether there was an additional effect of chemotherapy. N represents the number of patient in each study and is proportional to the size of the circle, and R represents the study reference number.



Logistic regression:

Variable	Odds ratio	Lower 95% CI	Upper 95% CI	p-value
Dose	1.15	1.06	1.24	0.001
Chemotherapy				
No	1	-	-	-
Yes	1.80	1.04	3.15	0.035

Figure 4.4. Scatter plot of response rate versus administered activity (AA) per Kg to assess the existence of a dose response relationship and the effect of chemotherapy in addition to ^{131}I-mIBG therapy. For each study N represents the number of patients and is proportional to the size of the circle, and R represents the study reference number.



Multivariate analysis, outcome is proportion

Variable	Odds ratio	Lower 95% CI	Upper 95% CI	p-value
Dose	0.52	0.21	1.30	0.164
Chemotherapy				
No	1	-	-	-
Yes	0.66	0.32	1.36	0.256

Comparative studies

Four studies had control arms and all were in the relapsed and refractory patients. No control group had been chosen by randomisation. In *Hutchinson et al. (1992)* investigators matched patients with neuroblastoma who had received chemotherapy consisting of ifosfamide and etoposide with patients who had received ^{131}I-mIBG. The control group in *Klingebiel et al. (1991a)* was made up of patients who had refused ^{131}I-mIBG therapy. In *Schmidt et al. (2006*), the controls came from patients whose local paediatric oncologist and nuclear medicine specialist decided against ^{131}I-mIBG treatment. In *Miano et al. (2001)* the control patients were not mIBG avid at ^{123}I-mIBG scanning suggesting that they may have had neuroblastoma with different biological behavior. Information as to the treatment patients received instead of ^{131}I-mIBG was not given (*Klingebiel 1991a*, Miano 2001, *Schmidt 2006).*

None of the studies presented tumour response data for the control groups. Survival outcomes were similar between ^{131}I-mIBG patients and controls across three studies, with median survival around 6 months in *Hutchinson et al. (1992)* and 15 months in *Klingebiel et al. (1991a).* By undertaking a multivariate Cox regression analysis, *Schmidt et al. (2006)* found a statistically significant difference in OS and EFS, ascribing the difference to the ^{131}I- mIBG group receiving ASCT, though the Cox analysis also pointed towards an increase in OS related to external beam radiation therapy and MYCN status.

Miano et al. (2001) reported that patients in the ^{131}I-mIBG group had a longer time to progression of 18 versus 3 months.

4.4 Discussion

This systematic review aimed to assess the activity and effectiveness of ^{131}I-mIBG therapy and to assess the overall quality and reliability of the current evidence base. The review also examined whether there was any evidence of a dose-response relationship and whether there was any additional value of concomitant chemotherapy.

No randomised controlled trials were identified comparing either ^{131}I-mIBG molecular radiotherapy with other treatments or different ^{131}I-mIBG schedules. We did find four studies that had non-randomised comparators, but believe that confounding variables, especially patient selection, make the validity of these comparisons highly doubtful. We therefore were unable to ascertain the relative effectiveness of ^{131}I-mIBG in the treatment of neuroblastoma.

Most studies assessed the activity of ^{131}I-mIBG using tumour response as an outcome measure. Three evaluated ^{131}I-mIBG during induction and consolidation. The majority of studies looked at its use in patients with relapsed or refractory disease, though only a third of the studies gave separate results for these two distinct groups, limiting the value of the data as these two groups are likely to respond differently.

Objective tumour rates of response to ^{131}I-mIBG therapy were of clinical significance, but there was no evidence that these responses lead to better long-term outcome as measured by EFS or OS.

It remains unclear whether or not there is a true dose response relationship. For the studies that reported cumulative AA we found a statistically significant association between higher cumulative AA and response rates (OR 1.15). For the studies that reported AA/kg, no statistically significant association was found, though the point estimate for the OR (0.52) was in the opposite direction. These divergent results suggest that chance or confounding factors may have contributed to these findings. The regression analysis on the studies that reported cumulative AA found a statistically significant relationship between tumour response and chemotherapy (OR 1.80). In the studies reporting AA/kg, it was not statistically significant, the point estimate for the OR (0.66) being in the opposite direction. We were unable to directly compare studies with and without chemotherapy as AA of ^{131}I-mIBG was reduced when given with chemotherapy.

Heterogeneity of the populations, ^{131}I-mIBG treatment schedules and other treatments will have influenced the tumour response results. Although most studies used tumour response measures equivalent to the INRC (Brodeur 1988 and 1993) [Table 4.6], there was variation in the timing of tumour response measurements. Given the inconsistency of reporting regarding key prognostic factors, it is impossible to determine the influence of confounding factors on this dose response result. ”Temporal drift‟ may also compound the problem (*Tang 2010*).

Similarly, the survival data also showed a wide spectrum of results and again may well be a reflection of the clinical heterogeneity between studies, with further confounding by subsequent treatment, therefore the true effect of ^{131}I-mIBG on survival is unknown.

The only significant acute toxicity reported was, as expected, myelosuppression. Haematopoietic stem cell support allows circumvention of this, and the safe use of higher AA. Few papers report long-term toxicities, but hypothyroidism and secondary thyroid cancer and myelodysplastic have been encountered.

We chose to use systematic review methodology because systematic reviews - by defining the scope of the review, setting out clear study objectives a priori, performing reproducible searches for studies, and performing tasks such as study selection and data extraction according to set criteria and in duplicate - ensure that bias in the selection of studies and selection of data summarized is minimized. This has enabled us to present a comprehensive, summary of the research evidence to date on the use of ^{131}I-mIBG in neuroblastoma. One consequence of this is that we identified multiple publications reporting the same patient group. This shows that the evidence base is in fact smaller than seems at first to be.

If we had assumed that the eight publications identified in induction were separate studies we would have 148 patients instead of 57. For relapsed and refractory studies, counting each publication as a separate study would have given 339 extra

patients. Multiple publications made data extraction difficult, and it could be that we may have double counted some patients or reduced the data set by mistakenly thinking some studies were related when they were not. By only including studies with 10 or more patients [Figure 4.1] will also have reduced the number of potential patients. This is an arbitrary cut off point often used in systematic reviews to save time critically appraising studies yielding very little information, or reporting exceptional cases which wouldn't be generalizable. In rare diseases this cut off point may be too high.

Systematic reviews also undertake quality assessment of the included studies, allowing for the review authors to to assess the validity of the results of each study, and thus the reliability of the review's summary findings. Selection bias was the most common problem, which along with the heterogeneity of the administration of ^{131}I- mIBG and the response assessment in the different studies, plus the small size of many studies which could lead to chance effects; these factors are probably the cause of the very wide range of response rates observed. We may be criticized that we have presented these data quantitatively, but we have presented the data in this way to illustrate the challenges faced by anyone trying to make sense of the many publications in this field.

Although we strongly advise caution in its interpretation, the data trend suggest that ^{131}I-mIBG is an "active" treatment in the short term, but provide no evidence on either its comparative efficacy relative to other therapeutic options or its long term benefits; and this enables us to justify the need for RCTs.

The currently available literature leads us to suggest that the greatest uncertainties are:

- How effective is 131I-mIBG compared to chemotherapy regimens (including high-dose therapy)?
- Is there a benefit of concomitant chemotherapy compared to giving 131I-mIBG alone?
- Is there an AA response relationship – i.e. what is the optimum dose?

We suggest that future trials should also take account of the following:

- There should be separate trials for relapsed and refractory patients; or at-least the two populations should be stratified and reported separately if included in the same trial.
- Fixed AA should be replaced by scaling to some proxy of size such as weight, body surface area, or prescribed to give a desired whole body dose, given the heterogeneity of the patient population.
- The resulting whole-body and tumour doses should be measured and reported to evaluate correlation between these parameters and response and other outcomes.

Over the last almost 30 years, many studies have demonstrated the activity of ^{131}I-mIBG therapy in neuroblastoma. There have however been no randomized controlled trials of its use and therefore its effectiveness compared with other

treatments remains unknown. There is too little data to assess whether it has efficacy during induction and consolidation. The true value of higher than standard AA, or increased cumulative AA, remains to be confirmed, as does the use of concomitant chemotherapy.

Chapter 5

Radiation doses received by comforters and carers during paediatric molecular radiotherapy

5.1 Introduction

Paediatric molecular radiotherapy can cause a great deal of anxiety for parents and other caregivers when thinking about radiation exposure. This study examines the radiation doses received by adults whilst looking after children receiving molecular radiotherapy. When adult patients are in hospital receiving molecular radiotherapy they are generally well and self-caring. However children, especially younger ones and babies, may require considerable personal care and emotional support from adults. Radioactive patients, and radioactive bodily products, including urine and vomit, and in the case of iodine-131 sodium iodide (^{131}I-NaI) also sweat, saliva and faecal matter, represent a potential radiation hazard to those caring for children receiving molecular radiotherapy.

Radiation exposure is governed by national legislation, which varies from country to country. In the United Kingdom, hospitals are bound by the Ionising Radiation Regulations 1999 (IRR99) which are derived from the European Union's Basic Safety Standards Directive 1996 96/29 (*Council Directive 96/29/Euratom of 13th May 1996*) as supplemented by the EU Medical Exposure Directive 1997 97/43 (*Council Directive 97/43/Euratom of 30th June 1997*). These regulations require that all radiation exposures shall be justified and optimized to a level, which is 'as low as reasonably achievable' (the ALARA principle).

The direct involvement of doctors and nurses in the period immediately following administration of the molecular radiotherapy agent is essential if radioactive

patients require specific medical or nursing interventions, and in carrying out these duties they will inevitably be exposed to some radiation. Staff may over time look after many patients. Therefore, respecting annual dose limits, and in keeping with the ALARA principle, it is essential that they do not receive any avoidable radiation exposure. To minimize staff exposure, normal child-care tasks such as feeding, washing, dressing, comforting and entertainment are delegated to other responsible adults during molecular radiotherapy.

These responsible adults are called *comforters and carers* and are usually family members, most often the parents, and less frequently other relatives such as grandparents, aunts, uncles or older siblings. '*Comforters and carers'* are defined by UK IRR99 as individuals 'knowingly and willingly helping (other than as part of their occupation) in the support and comfort of patients undergoing medical diagnosis or treatment' (*Ionising Radiation Regulations 1999 (IRR99) and European Union's Basic Safety Standards Directive 1996 96/29*).

Comforters and carers, unlike healthcare workers, are not subject to a specific cumulative dose limit, although incidental radiation exposure is governed by the ALARA principle. The International Commission on Radiological Protection (Publication 103) recommends the use of dose constraints rather than dose limits for *Comforters and Carers*. They suggest a dose constraint of 5 mSv per episode (i.e. for the duration of the risk of radiation exposure after therapy) but state that these constraints need to be used flexibly. They give the example that higher doses may well be appropriate for parents of very sick children. The ICRP 2007's

recommendations are endorsed by the UK Health Protection Agency (previously the UK National Radiological Protection Board).

Comforters and carers dose constraints are an important means of planning effective ways of reducing unnecessary exposure to these individuals. The UK HSE Approved Code of Practice to IRR99 (L121) states that it should always be appropriate to use dose constraints for *comforters and carers* and states that in most circumstances, it should be possible to design control measures that result in doses less than this. It also states that *comforters and carers* are likely to receive 1mSv or more in a year resulting from direct radiation or contamination during the comfort and support they offer.

In order to be designated as a *comforter and carer* for a child undergoing a medical exposure to radiation, it is necessary for that individual to be fully informed of the fact that he or she will be exposed to radiation, and given clear expectation of the risk of harm this may cause. In our institution, when a child is being assessed for molecular radiotherapy, potential comforters and carers are given a full verbal explanation together with a written information leaflet. *Unsealed source radiotherapy: information for comforters and carers*. The leaflet includes the following statement on risk:

> There is no legal limit to the amount of radiation dose you are allowed to receive as a comforter and carer. Consequently you may receive more radiation dose than is allowed for a member of the public. It is thought that exposure to even a small amount of radiation may result in a small increased chance of developing cancer in later years. For instance, the chance of harm resulting from the radiation dose a normal member of

the public is allowed to receive is 1 in 20,000. This chance of harm is similar to:

- Smoking 40 cigarettes during a lifetime.
- Driving a car for one year.
- Nine months of normal home life.

The chance that radiation will cause harm increases with the amount of radiation dose received. We will therefore aim to limit your radiation dose to no more than 5 times the public radiation dose limit. This is about twice the annual radiation dose we each receive from natural sources of radiation that exist in our normal environment. It is also less than the radiation dose a member of staff is legally allowed to receive.

Individuals are taught how to minimize their personal radiation exposure. This involves both avoidance of direct contamination by contact with radioactive materials, and minimizing exposure to the radiation emanating from the patient. Disposable plastic gloves, aprons and overshoes are used to reduce the risk of direct contamination by patient excreta – in the form of vomit, urine, sweat or faeces. Eating and drinking by *comforters and carers* in the ensuite inpatient bedroom is prohibited to reduce the risk of ingestion of radioactive substances. Individuals are advised to minimize the time spent in close contact with the child they are giving care to, to maximize the distance between themselves and the child when close contact is not necessary, and to use mobile, shielded protection screens where possible. Again, this advice is given verbally and re-iterated in the information leaflet. If the individual agrees to be a *comforter and carer*, written consent is then required. By signing the consent form the person certifies:

I am over 18 years of age.

I have been informed that, to give the extended support I want to provide to my relative/friend undergoing treatment with radioactive material, I am likely to receive a dose of ionising radiation in excess of the legal limit for a member of the public.

I have been informed about the possible risks involved with the exposure I may receive.

I have understood the instructions that specify how my dose may be restricted as far as reasonably practicable.

And for females aged between 12 and 55:

I understand that I should not be pregnant or breastfeeding whilst being a Comforter/Carer and that if there is any possibility of this I should inform the doctor/radiographer/nurse before becoming a Comforter or Carer.

Comforters and carers may be exposed to radiation both during that child's stay in hospital and after discharge. The period in hospital is the time of greater risk, as their *in vivo* activity is much higher. On discharge from hospital, patients are provided with a yellow, radionuclide instruction card detailing the molecular radiotherapy that was given and precautions, including a time frame, to be taken to reduce the exposure of other individuals in particular pregnant women and young children. These restrictions and advice are based on the dose rate measurements, the effective half-life of the isotope given, and take into account individual personal circumstances (*Working Party of the Radiation Protection Committee of the British Institute of Radiology 1999, Institute of Physics and Engineering in Medicine with the support of National Radiological Protection Board, Health and Safety Executive, The Health Departments and The Environment Agencies 2002*).

The medical use of radioactive substances can arouse anxiety in people who may misunderstand and overestimate the associated risks. This fear of radiation may affect both the relatives of patients, and even hospital staff who have been trained in radiation protection.

This work includes data on comforters and carers looking after relapsed or refractory neuroblastoma patients receiving ^{131}I-mIBG or ^{177}Lutetium DOTATATE therapy. I have also included in this work comforter and carer doses for the few patients who have received ^{131}I-mIBG or ^{177}Lutetium DOTATATE for neuroendocrine tumours and also those comforters and carers looking after patients receiving ^{131}I-NaI for thyroid carcinoma.

Thyroid cancer in children is rare. Data from the European database of the Automated Childhood Cancer Information System (ACCIS) reported age-standardised incidence rates for children aged 0-14 years varied in European regions from 0.5 to 1.2 per million and age-specific incidence for 1-19 year olds varied between 4.4 to 11 per million (*Steliarova-Foucher 2006*). In England and Wales the annual incidence is 0.5/million/year. Thyroid cancers occurring under the age of 21 years accounts for <10% of thyroid cancer. Children commonly present with advanced disease with 40-80% having regional lymph node involvement at diagnosis and 10-20% having metastases, usually lung, at presentation (Papendieck 2011*, Dinauer 2008*). Despite this, the prognosis for children with thyroid cancer is excellent with mortality rates of less than 10% (*Stelliarova-Foucher 2006, Alessandri 2000*). The presence of metastases requires repeated administrations of ^{131}I-NaI, usually given 6 months

apart. ^{131}I-NaI molecular radiotherapy has an established role in the effective treatment of paediatric thyroid cancer and several studies have shown that successful treatment with ^{131}I-NaI may be due to the higher expression of the sodium iodide symporter (*Patel 2002, Faggiano 2004*). It is therefore important that the comforters and carer's doses for this group of patient's is monitored over time.

This study examines the in-hospital radiation exposure of *comforters and carers*, to see whether these were within the established dose constraint. The aim was to provide real data that might help to reassure potential *comforters and carers*, and to better educate hospital staff, who might otherwise seek to restrict reasonable parental access to children undergoing molecular radiotherapy, and cause emotional distress to both patients and carers, in a misguided attempt to reduce the risks.

5.2 Materials and Methods

University College London Hospitals NHS Foundation Trust is a UK national referral centre for paediatric molecular radiotherapy. We use three different types of molecular radiotherapy in children: iodine-131 meta-iodobenzylguanidine (^{131}I-mIBG) and lutetium-177 DOTATATE (^{177}Lu-DOTATATE) for neuroblastoma and other neuroendocrine tumours, and ^{131}I-NaI for thyroid cancer.

In neuroblastoma, we used a protocol that requires higher than standard activities of ^{131}I-mIBG in two administrations with concurrent topotecan as a radiosensitiser, (mIBG and topotecan in neuroblastoma – the MATIN protocol), two weeks apart, followed by peripheral blood stem cell support. We used a weight-based activity of 444 MBq kg^{-1} for the first administration, aiming to give a whole body radiation absorbed dose of about 2 Gy. The actual whole body dose achieved was determined via the whole body monitoring of radionuclide retention using monitors installed in the inpatient bedroom. The activity required for the second administration to deliver a total whole body dose for both administrations of 4 Gy was then calculated (Gaze 2005). Patients with neuroendocrine tumours and some patients with neuroblastoma received a single weight based administered activity of 444MBq kg^{-1}.

The administered activity of ^{177}Lu-DOTATATE was a fixed administered activity of 7.4GBq (*Gains 2011*). Fixed standard administered activities of ^{131}I-NaI were used in thyroid cancer patients: 3.0 GBq for ablation, and 5.5GBq for therapy of residual or metastatic disease.

Potential *comforters and carers* were given verbal and written explanation on the radiation risks and methods to reduce their radiation exposure. Methods to reduce their radiation exposure such as time, distance and use of shielding, as well as methods to avoid contamination such as gloves, aprons and overshoes were explained. Informed consent to be a *comforter and carer* was then obtained. The designated *comforters and carers* were given an instant readout whole body personal dosimeter ('Bleeper Sv' from Vertec Scientific Ltd, Silchester, UK) to use for the duration of the patient's stay in hospital. They were instructed in its use, and asked to record their exposure following each contact, in a written log kept outside the patient's room.

The HPA advised dose constraint is 5mSv per episode, for the duration of a given release of therapy. Therefore an episode, for this study, was defined as a single administration of ^{177}Lu-DOTATATE or ^{131}I-NaI. For the ^{131}I-mIBG patients, an episode was defined as either a single high-administered activity treatment schedule (MATIN - 2 administrations of ^{131}I-mIBG, 2weeks apart) or a single administration of ^{131}I-mIBG.

We collected the *comforter and carer* data for paediatric molecular radiotherapy episodes between November 2002 and March 2012. We then compared the recorded *comforter and carer* exposures against national and international recommendations for the applied dose constraint. We looked for associations between the radionuclide, the administered activity received and age of the patients; the comforter and carer exposure, the administered activity and the age of

the patients, and the total number of *comforters and carers* per child. Data are presented descriptively and linear regression analysis was used to test for significance of associations.

5.3 Results

For ^{131}I-mIBG, the MATIN protocol was used in 57 instances and there were 12 single administrations of ^{131}I-mIBG. The MATIN protocol was used in patients with refractory or relapsed neuroblastoma. The 12 single administrations of ^{131}I-mIBG were used in nine instances of neuroblastoma and three for a neuroendocrine tumour. *Comforter and carer* data were available for all the single administrations of ^{131}I-mIBG, and for 50 of the 57 MATIN administrations (88%). Twenty-four administrations of ^{177}Lu-DOTATATE (18 for neuroblastoma and six for other neuroendocrine tumours) were given. Data were available for 15 of these administrations (72%). Forty-one administrations of ^{131}I-NaI for thyroid carcinoma were given. Data were available for 28 (68%) of these administrations.

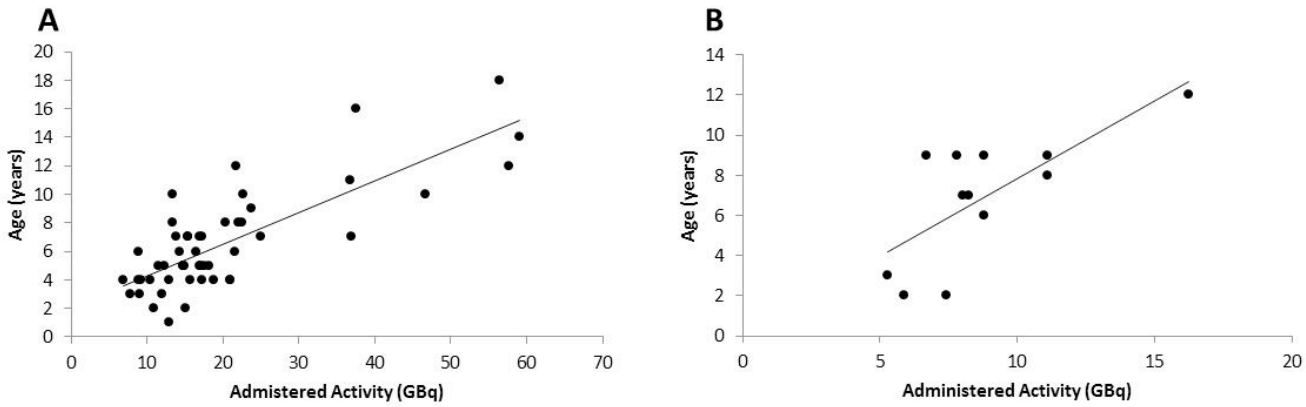
The median age of the children for all administrations was 7 years (range 1-18 years). For patients receiving ^{131}I-mIBG the median age was 6 years (range 1-18 years); ^{177}Lu-DOTATATE 7 years (range 2-14) and ^{131}I-NaI median 9 years (range 5-17 years).

The median administered activity of ^{131}I-mIBG per MATIN was 16.2GBq (range 6.8-59GBq) and for a single administered activity of ^{131}I-mIBG was 8.1GBq (range 5.26-16.25GBq). The median administered activity of ^{177}Lu-DOTATATE per administration was 7.2GBq (range 2.5-7.5GBq). For thyroid carcinoma patients receiving ^{131}I-NaI, 11 administrations were for ablation at a fixed administered activity of 3GBq and 17 were therapy doses given at a fixed administered activity of 5.5GBq.

The median number of *comforters and carers* per ^{131}I-mIBG MATIN course was two (range one to nine); single administration of ^{131}I-mIBG median two (range one-five); ^{177}Lu-DOTATATE median one (range one-two); ^{131}I-NaI median two (range one-two).

For ^{131}I-mIBG patients the administered activity increased with increasing age for both the MATIN (Figure 1.5A) and single administered activity patients (Figure 5.1B) as the administered activity prescribed was weight based.

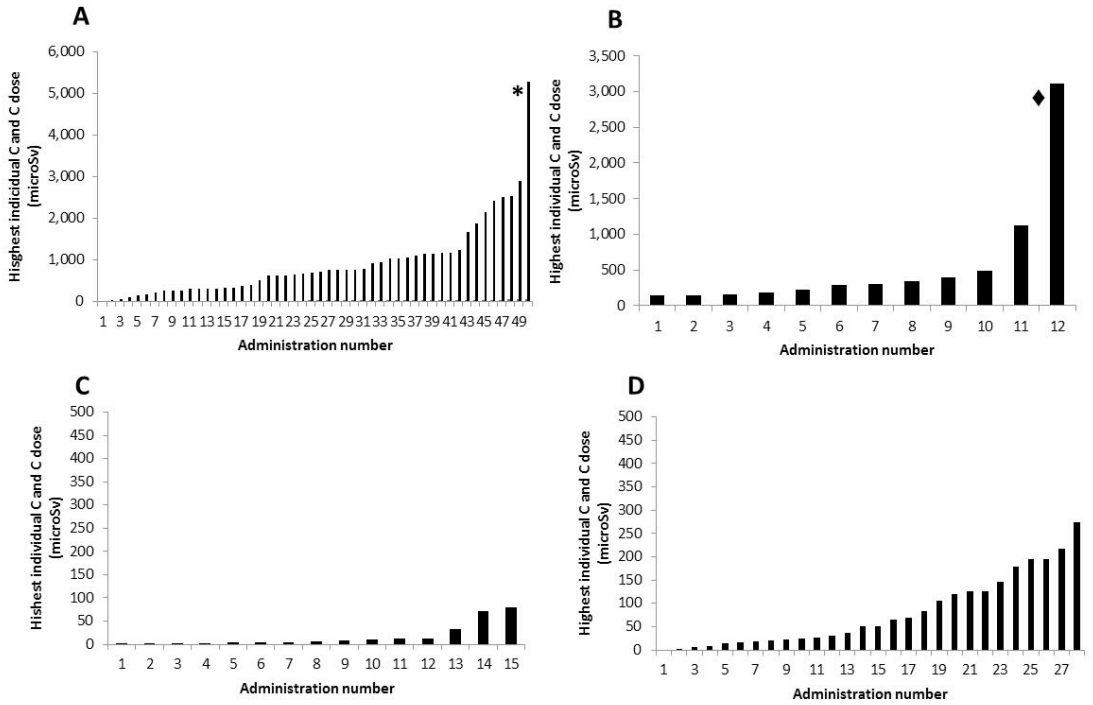
Figure 5.1 The administered activity (GBq) increased with the patient's age for both the MATIN (Figures 5.1A)(R2=0.6201, p<0.001) and single administration of ^{131}I-mIBG patients (Figure 5.1B) (R2=0.5217, p=0.008).



There was no correlation between the administered activity and the age for the ^{177}Lutetium DOTATATE and ^{131}I-NaI patients as fixed administered activities were used.

The median *comforter and carer* exposure for a single course of MATIN was 302 µSv (range 0 – 5,282 µSv) and for a single administration of ^{131}I-mIBG was 163 µSv (range 3-3,104 µSv); for ^{177}Lu-DOTATATE the median exposure per administration was 6 µSv (range 1-79 µSv); for ^{131}I-NaI the median exposure per administration was 37 µSv (range 0-274 µSv) (Figure 5.2).

Figure 5.2 A-D show the highest individual comforter and carer dose per administration of MATIN (Figure A), a single administration of ^{131}I-mIBG (Figure 5B), ^{177}Lutetium DOTATATE (Figure C) and ^{131}I-NaI (Figure D).



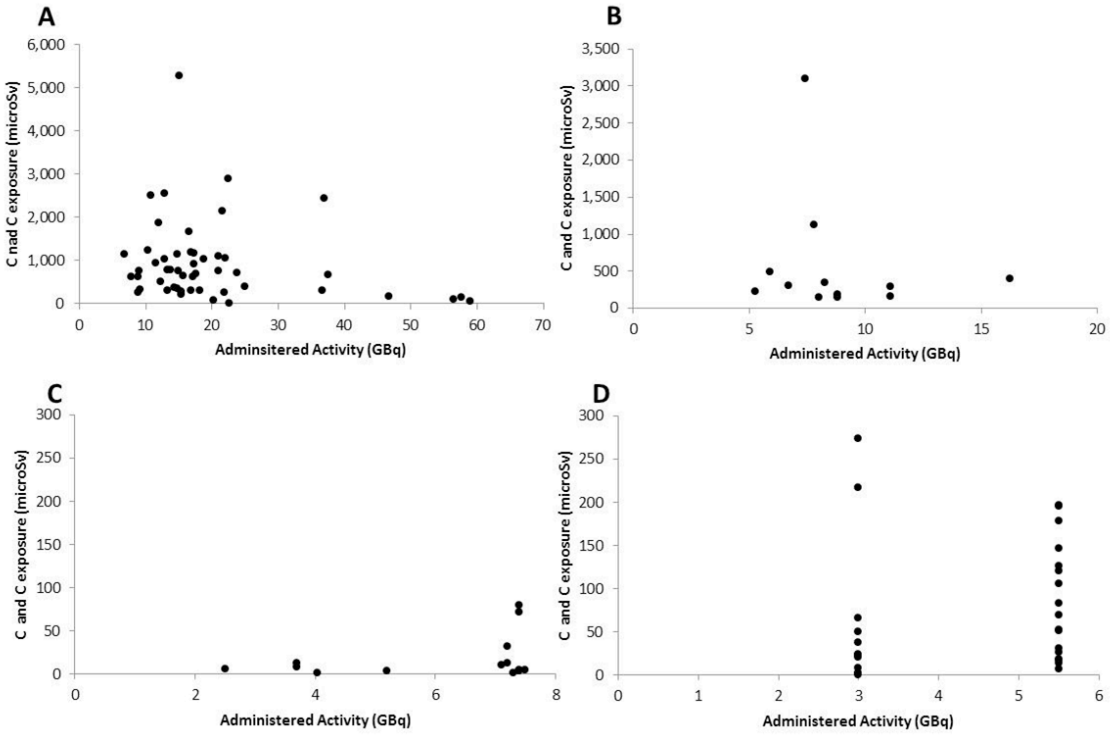
* This patient was 2 years old, in nappies and visually impaired.

♦ This patient was only 2 years old and in nappies.

For MATIN patients: 1/50 > 5mSv, 16/50 >1mSv and <3 mSv; 33/50<1mSv. Highest individual comforter and carer dose for the single ^{131}I-mIBG administrations: 2/12 >1mSv; 10/12 <1mSv. Highest individual comforter and carer dose for the ^{177}Lu-DOTATATE and ^{131}I-NaI administrations: all *comforters and carers* were below 1mSv.

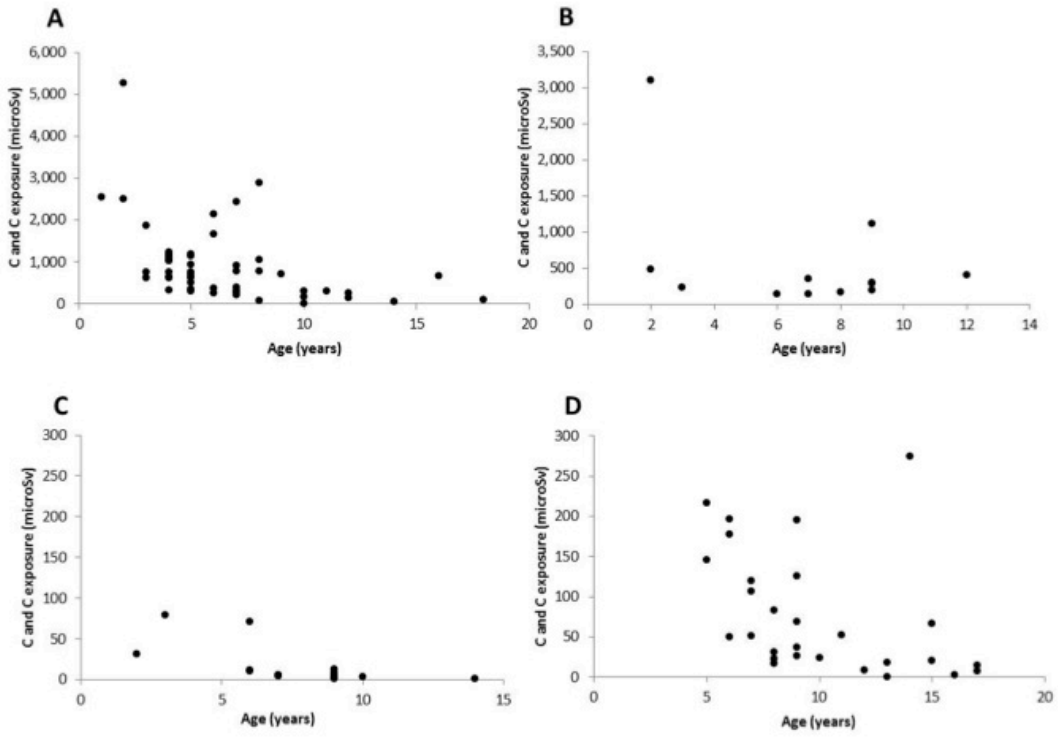
Over the 10-year period examined, only one comforter and carer had a measured in-hospital radiation exposure greater than the dose constraint of 5mSv per course of treatment as proposed by the Health Protection Agency. In 86% of cases the comforter and carer dose was <1mSv.

Figures 5.3 A-D display the highest individual *comforter and carer* exposure (equivalent dose in μSv) and the administered activity (GBq) for each of the four different treatment schedules.



For the ^{131}I-mIBG patients (both MATIN and single administered activity) the highest administered activities did not result in higher *comforter and carer* doses. For the ^{177}Lu-DOTATATE and ^{131}I-NaI patients, a fixed administered activity was used, and the graphs show a range of comforter and carer doses for the same fixed administered activity.

Figure 5.4 shows the correlation between the age of the child and the highest individual *comforter and carer* exposure for each of the different therapies. For both the MATIN and single administration of ^{131}I-mIBG patients the highest *comforters and carer* doses were seen in the youngest patients and the lowest in the older patients (A and B). This was also true for the majority of cases in the ^{177}Lu-DOTATATE patients (C) and the ^{131}I-NaI patients (D); there was one older patient with a higher comforter and carer dose (D).



5.4 Discussion

This study reports on 10 years of *comforter and carer* exposure data for ^{131}I-mIBG, ^{177}Lu-DOTATATE and ^{131}I-NaI. There were higher comforter and carer doses for the ^{131}I-mIBG groups than the ^{177}Lu-DOTATATE or ^{131}I-NaI groups as would have been expected due to significantly higher administered activities being used.

The greatest proportion of our data came from *comforters and carers* looking after patients receiving ^{131}I-mIBG molecular radiotherapy. The administered activity in these patients is weight based, and therefore the administered activity increases with increasing age as older patients are usually heavier (Fig.5.1A and 5.1B). Although the older patients got significantly higher administered activities, this did not result in higher doses to the *comforters and carers* as these older patients required less input and support. The highest *comforter and carer* doses were seen in the younger patients (Fig. 5.4A-D). The two patients with the highest individual comforter and carer doses (5282µSv and 3104 µSv) were in cases where the child was 2 years of age, still in nappies and in the highest case the patient was also visually impaired, requiring more care, therefore explaining the higher exposure received (Figure 5.2A and 5.2B).

The *comforter and carer* doses from the ^{177}Lu-DOTATATE and ^{131}I-NaI administrations were much lower then for ^{131}I-mIBG administrations and showed a range of doses received for the same fixed administered activity (Figure 5.2C and

5.2D). The comforter and carer doses were again, in the majority of cases, lower for the older patients reflecting the less input required for this age group.

There was no correlation between the number of *comforters and carers* and the highest individual or total *comforter and carer* dose. The majority of patients had 2 *comforters and carers* and the number of patients with more *comforters and carers* was in comparison very small.

Molecular radiotherapy has an increasingly significant role to play in the treatment of several paediatric malignancies including thyroid carcinoma and neuroblastoma. To enable the successful delivery of molecular radiotherapy to children, teenagers and young adults it is essential that they are allowed comfort and support from relatives and other care givers during their treatment, but there is often anxiety amongst the public, and staff, in caring for paediatric patients receiving molecular radiotherapy, especially when higher than standard administered activities are used.

There are minimal data in the literature on *comforter and carer* doses during paediatric molecular radiotherapy. With regards to ^{131}I-mIBG therapy there have been two published papers on parental exposures. One included three patients and the other only one (*Van der Steen 1986, Tristam 2001*). Published data on doses to family members during ^{131}I-NaI therapy is on spouses and partners during the outpatient phases of their treatment and not on *comforters and carers* looking after paediatric patients who have received treatment (*Harbet 1974, Thomson 1995, Monsieurs 1998, Mathieu 1999*). Barrington *et al*. have published the internal doses

received by family members of outpatients treated with ^{131}I for the treatment of hyperthyroidism (*Barrington 1999, 2008*).

The published report 'Dose constraints for comforters and carers' produced by Royal Hallamshire Hospital for the Health and Safety Executive includes small amounts of contributed but unpublished data on parental radiation exposure from ^{131}I-NaI and ^{131}I-mIBG therapy (*Singleton 2003*).

A recent publication by *Markelewicz et al.* reported on the radiation exposure to the parents and nursing staff for 14 neuroblastoma patients treated with ^{131}I-mIBG therapy. The administered activity of ^{131}I-mIBG ranged from 5.92GBq to 23.31GBq (mean 13.65GBq). All nurses and caregivers in this study received exposures of <5mSv during the in-patient hospital stay of the patient. No significant correlation was found between the caregiver or nursing exposure and the administered activity of ^{131}I-mIBG and no significant correlation was found with patient age (*Markelewicz 2013*).

Limitations of this study include a lack of knowledge of the radiation exposure to the *comforters and carers* following discharge home from hospital. Under our local rules, patients may be discharged with no restrictions when the residual radioactivity level has fallen to 30MBq of ^{131}I. Patients may be discharged with a residual level up to 150MBq ^{131}I provided contact with children or women who are or might be pregnant is avoided. Our recorded *comforters and carers* data represent exposure from

administration to discharge from hospital, and so these include the majority of loss of the radiopharmaceutical through excretion or decay. For example, a 20kg child receiving 444MBq/kg (total administered activity 8.88GBq) loses over 99% of radiation prior to discharge with a residual activity of 30MBq. Our recorded *comforter and carer* doses therefore represent a very significant proportion of the exposure extrapolated to infinity. In collecting the data we relied on *comforters and carers* accurately inputting the data and in the majority of cases this was very detailed. Overall we had *comforter and carer* radiation exposure data for 85% of our administrations over a 10 year period.

In conclusion, over a 10 year period, the absorbed radiation dose to *comforters and carers* during paediatric molecular radiotherapy with ^{131}I-mIBG, ^{177}Lu-DOTATATE and ^{131}I-NaI was in all but one case within the recommended dose constraints stipulated by the Health Protection Agency. Even when high administered activities are used we have shown that with the appropriate information, education and guidance, dose constraints for comforters and carers can be respected. For the high-dose ^{131}I-mIBG patients, the main determinant of a high *comforter and carer* dose was the age of the child and nappy requirements rather than the high administered activities as may have been expected and so we recommend particular caution in this group of patients.

Chapter 6

^{18}F-Fluorodeoxyglucose positron emission tomography scans performed before and after ^{131}I-*meta*-Iodobenzylguanidine therapy for high –risk neuroblastoma

6.1 Introduction

Functional imaging plays an essential role in the diagnosis, staging and response assessment of metastatic neuroblastoma. Over 90% of neuroblastomas express the noradrenaline transporter molecule and this enables imaging with the guanethidine derivative, mIBG.

Most commonly, mIBG is labeled with ^{123}I for imaging and ^{123}I-mIBG scintigraphy has become the most frequent functional imaging modality for assessing metastatic disease in high-risk neuroblastoma. ^{123}I emits gamma photons only – principally at 159keV (83% abundance) and it has a physical half-life of 13.13 hours. These physical characteristics mean both that the use of ^{123}I results in better image quality, especially when using SPECT, and that it has a more favorable profile for radiation protection, than ^{131}I, which is preferred for therapy. ^{131}I principally emits gamma photons, 364keV (81% abundance), and also emits beta particles – 0.61MeV (max) and 0.192MeV (mean). It has a physical half-life of 8.04 days.

A recent report from the INRG task force has recommended guidelines for the performing of mIBG scans to allow standardization between institutions and facilitate their use within clinical studies (*Matthay 2010*). The report also recommended that mIBG scans should be scored by a semi-quantitative scoring system to reduce inter-observer variability and to improve the comparison of response rates between different clinical trials (see chapter 1).

Although mIBG remains the gold standard, it does have limitations. Approximately 10% of patients with neuroblastoma will have mIBG negative disease making it necessary to have other methods of disease assessment in these patients. ^{123}I-mIBG scintigraphy has limited spatial resolution and the ability to accurately quantify results is challenging. Semi-quantitative scoring systems of ^{123}I-meta-iodobenzylguanidine (mIBG) scans for response assessment in neuroblastoma have been validated and give a good quantification of anatomical disease extent. The extent of disease on mIBG scanning before and after treatment has been shown to be of prognostic significance (see chapter 1).

^{123}I-mIBG scans often require correlation with radiological examinations, either CT or MRI, especially in cases of uncertain uptake. The introduction of SPECT (single photon emission tomography), and co-registration with CT images acquired as part of the same investigation, can improve the visualisation of small foci of neuroblastoma that can be difficult to detect on planar scintigraphy, and their anatomical localization, but it is not always performed at present, and the additional information which may be gained is not used in current semi-quantitative scoring systems.

The challenges mentioned above may to some extent be overcome with ^{124}I-mIBG PET/CT, but this functional imaging modality is only just entering early phase clinical trials within the UK and will not be a solution for the 10% of patients who are mIBG negative.

^{18}F-FDG PET makes use of a glucose analogue, flurodeoxyglucose (FDG), labeled with the positron emitting isotope of fluorine, ^{18}F. ^{18}F-FDG, like glucose itself, is actively transported from the bloodstream into cells with high metabolism. It is retained within them in its phosphorylated form, as lacking the 2'-hydroxyl group it cannot, like glucose, undergo normal glycolysis. The degree of uptake of FDG is proportional to the tumour cell metabolism and the tumour burden of the patient. Positron emission tomography can be used to map areas of high ^{18}F-FDG accumulation within the body, at sites of high metabolism including normal structures like the brain, brown fat and the heart, and also in deposits of metabolically active cancers. Co-registration with CT imaging, acquired as part of the same investigation, allows reporting physicians to see exactly where in the body the tracer is localized. ^{18}F-FDG PET/CT therefore has a unique ability to give anatomical and metabolic information and has established its role in staging and response assessment for many adult tumours. For example, in the early response assessment of lymphoma patients, by demonstrating those which have responsive disease, it helps to risk stratify the disease, and thereby guide the intensity of further treatment (*Hutchings 2006, Jerusalem 2001*).

The precise role of ^{18}F-FDG PET/CT in neuroblastoma response assessment has not been defined and the methods used not standardised. The current INRC (*Brodeur 1993*) does not include semi-quantitative scoring of mIBG scans or other functional imaging modalities such as ^{18}F-FDG PET/CT, but these are likely to be incorporated in future revisions. The methods for response assessment of ^{18}F-FDG PET in neuroblastoma have also not been established and vary in the limited reports in the

literature *(Shulkin 1996, Sharp 2009, Taggart 2009, Melzer 2011, Papathanasiou 2011).*

This study compares two different methods of response assessment for ^{18}F-FDG PET/CT in neuroblastoma, and evaluates whether ^{18}F-FDG PET/CT imaging adds additional information to that gained from ^{123}I-mIBG scintigraphy in the response assessment of neuroblastoma patients treated with ^{131}I-mIBG.

6.2 Materials and Methods

Patients with relapsed or refractory neuroblastoma who had received high-administered activity ^{131}I-mIBG therapy with concurrent Topotecan (the MATIN protocol) *(Gaze 2005)* between 2006 and 2010 at University College London Hospitals NHS Foundation Trust were retrospectively identified. Eligible patients for this study were required to have paired ^{123}I-mIBG and ^{18}F-FDG PET/CT scans both pre- and post-high dose ^{131}I-mIBG therapy.

All scans were performed within the same institution with standardised imaging protocols. Prior to ^{123}I-mIBG imaging, all patients received thyroid blockade by oral administration of Potassium Iodide. In addition, all other medicines known to interfere with tumour uptake of radiolabelled mIBG were discontinued. All patients were imaged on a GE Infinia Hawkeye 4 gamma camera system. Imaging was performed at 4 and 24 hours post-injection. Whole-body images were obtained at both time-points and SPECT/CT images acquired immediately after the 24 hour whole body image. Whole-body images were obtained using low-energy high-resolution collimators with a 20% energy window centred over a 159keV photopeak. SPECT/CT images were acquired with an additional scatter window centred over 130keV. Projection time was 40s and 120 projections acquired, with the region to be evaluated assessed from the patient history and whole-body image. Localisation was aided with a planar x-ray scout view. Following SPECT, low-dose CT was acquired with slice thickness of 1mm. Data was reconstructed using iterative reconstruction

(20 iterations) with attenuation correction derived from CT. Scatter correction was also applied.

For the ^{18}F-FDG PET/CT imaging, all patients were imaged on a Discovery STE PET/CT system (GE Medical Systems). Patients were asked to fast for 6 hours prior to imaging. A minimum of 14MBq to a maximum of 400MBq ^{18}F-FDG was administered with an uptake time of 50 to 75 minutes. Whole body images were acquired to define the axial range of the PET/CT study, covering the area from the top of the head to mid-thighs. A CT was acquired with pitch 1.5 and 5mm collimation. Tube voltage was 120-140kVp and tube current 80mA. PET was performed immediately after CT with the number of bed positions appropriate for the axial range. PET was acquired in 2D mode (5mins/bed position). PET was reconstructed using 2 iterations and 28 subsets with attenuation correction calculated from transmission maps derived from CT.

Scans were retrospectively analysed by two independent nuclear medicine physicians experienced in interpreting nuclear medicine imaging including ^{123}I-mIBG and ^{18}F-FDG PET/CT imaging.

The response on ^{123}I-mIBG scans was evaluated by a modified SIOPEN method of semi-quantitative scoring (*Lewington 2011*). mIBG semi-quantitative scores take in to account skeletal disease only. The skeleton is divided in to 12 segments and a score of 0-6 assigned for each segment therefore giving a total score out of 72 for the skeleton.

A soft tissue score was also added for the purpose of this analysis to map the full extent of the disease – the soft tissue compartments were divided into 8 segments with a score 0-2 assigned for each soft tissue segment giving a total score out of 16 for the soft tissue disease. An example of the semi-quantitative score sheet is shown in Figure 6.1.

Table 6.1 Semi-Quantitative Score Sheet

Skeleton Score				**Soft tissue score**	
	Pre treatment	Post Treatment		Pre treatment	Post treatment
Skull/facial bones			Head and Neck		
Thoracic cage			Thorax		
Right humerus			Right upper limb		
Left humerus			Left upper limb		
Right forearm					
Left forearm					
Spine			Abdomen		
Pelvis			Pelvis		
Right femur			Right lower limb		
Left femur			Left lower limb		
Right tibia/fibula					
Left tibia/fibula					
TOTAL	/72	/72	TOTAL	/16	/16

SKELETAL SCORE

0 No abnormality
1 1 focal lesion
2 2 focal lesions
3 3 focal lesions
4 Diffuse <50% of bone or >3 focal lesions
5 Diffuse 50-95% of bone
6 Diffuse involving whole bone
X Unevaluable

SOFT TISSUE SCORE

0 No abnormality
1 Solitary lesion
2 Multiple lesions
X Unevaluable

^{18}F-FDG PET scans were evaluated by two methods: a semi-quantitative score and by PET Response Criteria in Solid Tumours (PERCIST) (*Wahl 2009*). The semi-quantitative method uses maximum intensity projection (MIP) images only, exactly as per the above method for ^{123}I-mIBG scans. It therefore relies on the number and distribution of lesions identified, and does not take into account the degree of uptake – a single faint lesion scores the same as a single intense lesion of the same size. In contrast, the PERCIST system takes intensity into account by requiring SUV_{max} scores to be recorded for each anatomical segment with disease pre- and post-treatment with ^{131}I-mIBG.

Table 6.2 Semi-quantitative response criteria – for both ^{123}I-mIBG scans and ^{18}F-FDG PET/CT scans was defined as follows. The relative score of both scans was obtained by dividing the post-score by the pre-score.

Score (post score / pre score)	Response Grouping
0	Complete Response
0-0.1	Very Good Partial Response
0.1-0.5	Partial Response
0.5-0.75	Minor Response
0.75-1.25	Stable Disease
>1.25	Progressive Disease

Table 6.3 For the ^{18}F-FDG PET/CT scans a PERCIST score was also obtained by measuring the percentage change in SUV_{max} scores pre and post treatment with ^{131}I-mIBG molecular radiotherapy:

SUV_{max}	**PERCIST SCORE**
Score 0	Complete Metabolic Response
> 30% reduction in SUV_{max}	Partial Metabolic Response
Criteria not met for partial metabolic response or progressive metabolic response	Stable Disease
> 30% increase in SUV_{max}	Progressive Disease

6.3 Results

All patients were treated at University College London Hospitals NHS Foundation Trust between March 2006 and May 2010.

15 patients with neuroblastoma who had been treated with ^{131}I-mIBG molecular radiotherapy were identified who had paired ^{123}I-mIBG and ^{18}F-FDG PET/CT scans pre and post treatment. A total of 17 paired ^{123}I-mIBG and ^{18}FDG-PET/CT scans pre and post ^{131}I-mIBG therapy were identified as 2 patients had received ^{131}I-mIBG therapy twice (patient 4A/B and 13A/B).

13 of the patients had INSS stage 4 neuroblastoma and 2 patients INSS stage 3 disease. The median age was 5 years, range 1 to 18 years.^{131}I-mIBG was given in the relapse setting in 7 instances and for refractory disease on 10 occasions.

The ^{131}I-mIBG was given as per the MATIN protocol (mIBG and topotecan in neuroblastoma). ^{131}I-mIBG was given in two administrations, two weeks apart, with concurrent topotecan as a radiosensitiser. This was followed by peripheral blood stem cell support. A weight-based activity of 444 MBq kg^{-1} of ^{131}I-mIBG for the first administration was given, aiming to give a whole body radiation absorbed dose of about 2 Gy. The actual whole body dose achieved was determined via the whole body monitoring of radionuclide retention using monitors installed in the inpatient

bedroom. The activity required for the second administration was calculated to deliver a total whole body dose for both administrations of 4 Gy (*Gaze 2005*).

Table 6.4 Characteristics of the 15 patients studied. Patient 4 and 13 had two different therapy administrations with ^{131}I-mIBG (4A and 4B, 13A and 13B)

Patient No.	Age	Sex	INSS Stage	Relapsed/ Refractory	Time from ^{131}I-mIBG to f/U scan
1	18	M	4	Relapsed	23 weeks
2	2	F	4	Refractory	6 weeks
3	5	M	3	Relapsed	39 weeks
4A	3	M	4	Refractory	9 weeks
4B	3	M	4	Refractory	6 weeks
5	8	M	4	Relapsed	8 weeks
6	5	F	4	Refractory	17 weeks
7	5	F	4	Relapsed	8 weeks
8	12	F	4	Relapsed	8 weeks
9	14	M	4	Refractory	6 weeks
10	5	M	4	Refractory	5 weeks
11	7	M	4	Refractory	7 weeks
12	18	M	3	Refractory	11 weeks
13A	1	M	4	Refractory	5 weeks
13B	2	M	4	Refractory	7 weeks
14	7	M	4	Relapsed	12 weeks
15	7	F	4	Relapsed	8 weeks

Pre-treatment ^{123}I-mIBG and ^{18}F-FDG PET/CT scans were in all but one case performed within 3 weeks of the start of ^{131}I-mIBG therapy. Post-treatment ^{123}I-mIBG and ^{18}F-FDG PET/CT scans were performed at a median of 8 weeks (range 5 to 39 weeks) after high-administered activity ^{131}I-mIBG therapy.

The extent of disease on the pre-therapy ^{131}I-mIBG and ^{18}F-FDG scans was variable between the two imaging modalities as shown in table 6.5

Table 6.5 shows the semi-quantitative scores and distribution of disease on ^{123}I-mIBG and ^{18}F-FDG PET/CT before treatment with ^{131}I-mIBG.

PATIENT		mIBG Score Pre	FDG Score Pre	Distribution of disease ^{123}I-mIBG v ^{18}F-FDG PET/CT
1	Skeleton	7	3	^{123}I-mIBG > ^{18}F-FDG PET/CT
	Soft Tissue	0	0	
2	Skeleton	4	0	^{123}I-mIBG > ^{18}F-FDG PET/CT
	Soft Tissue	0	0	
3	Skeleton	11	11	^{18}F-FDG PET/CT>^{123}I-mIBG
	Soft Tissue	3	4	
4A	Skeleton	16	12	^{123}I-mIBG > ^{18}F-FDG PET/CT
	Soft Tissue	1	1	
4B	Skeleton	11	0	^{123}I-mIBG>^{18}F-FDG PET/CT
	Soft Tissue	1	1	
5	Skeleton	54	30	^{123}I-mIBG>^{18}F-FDG PET/CT
	Soft Tissue	2	2	
6	Skeleton	13	32	^{18}F-FDG PET/CT>^{123}I-mIBG
	Soft Tissue	2	2	
7	Skeleton	26	9	^{123}I-mIBG>^{18}F-FDG PET/CT
	Soft Tissue	0	1	
8	Skeleton	1	11	^{18}F-FDG PET/CT>^{123}I-mIBG
	Soft Tissue	3	8	
9	Skeleton	0	0	^{123}I-mIBG=^{18}F-FDG PET/CT
	Soft Tissue	4	4	
10	Skeleton	8	16	^{18}F-FDG PET/CT>^{123}I-mIBG
	Soft Tissue	0	1	
11	Skeleton	0	0	^{123}I-mIBG=^{18}F-FDG PET/CT
	Soft Tissue	2	2	
12	Skeleton	0	0	^{18}F-FDG PET/CT>^{123}I-mIBG
	Soft Tissue	1	3	
13A	Skeleton	0	0	^{123}I-mIBG=^{18}F-FDG PET/CT
	Soft Tissue	2	2	
13B	Skeleton	0	0	^{123}I-mIBG=^{18}F-FDG PET/CT
	Soft Tissue	2	2	
14	Skeleton	24	24	^{123}I-mIBG=^{18}F-FDG PET/CT
	Soft Tissue	1	1	
15	Skeleton	9	19	^{18}F-FDG PET/CT>^{123}I-mIBG
	Soft Tissue	0	0	

For mapping of disease extent prior to ^{131}I-mIBG therapy, the ^{123}I-mIBG scan showed more disease in 6 out of 17 cases; the ^{18}F-FDG scans showed more disease in 6 out of 17 cases; the ^{18}F-FDG PET/CT and ^{123}I-mIBG showed the same extent of disease in 5 out of 17 cases as shown in the pie chart below.

Figure 6.1

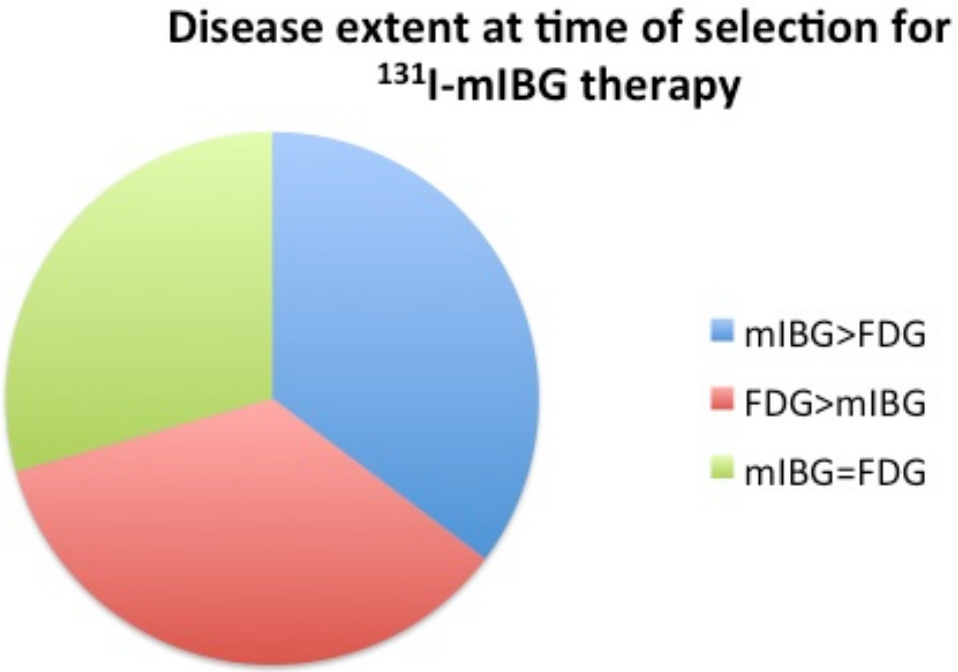


Table 6.6 Response on ^{123}I-mIBG and ^{18}F-FDG PET/CT by semi-quantitative scoring

PATIENT		mIBG Score Pre	mIBG Score Post	mIBG Relative Score	mIBG Response	FDG Score Pre	FDG Score Post	FDG Relative Score	FDG Response
1	Skeleton	7	0	0	CR	3	0	0	CR
	Soft Tissue	0	0	-		0	0	0	
2	Skeleton	4	0	0	CR	0	0	-	-
	Soft Tissue	0	0	-		0	0	-	
3	Skeleton	11	5	0.45	PR	11	10	0.9	PR
	Soft Tissue	3	3	1		4	2	0.5	
4A	Skeleton	16	11	0.7	MR	12	0	0	PR
	Soft Tissue	1	1	1		1	1	1	
4B	Skeleton	11	3	0.3	PR	0	0	-	CR
	Soft Tissue	1	1			1	0	0	
5	Skeleton	54	47	0.9	SD	30	18	0.6	PR
	Soft Tissue	2	1	0.5		2	1	0.5	
6	Skeleton	13	4	0.3	PR	32	0	0	PR
	Soft Tissue	2	2	1		2	2	1	
7	Skeleton	26	4	0.15	PR	9	0	0	PR
	Soft Tissue	0	0			1	1	1	
8	Skeleton	1	0	0	SD	11	3	0.3	PR
	Soft Tissue	3	3	1		8	4	0.5	
9	Skeleton	0	0	-	SD	0	0	-	SD
	Soft Tissue	4	4	1		4	4	1	
10	Skeleton	8	8	1	SD	16	0	0	VGPR
	Soft Tissue	0	0	-		1	1	1	
11	Skeleton	0	0	-	SD	0	0	-	SD
	Soft Tissue	2	2	1		2	2	1	
12	Skeleton	0	0	-	SD	0	0	-	SD
	Soft Tissue	1	1	1		3	3	1	
13A	Skeleton	0	0	-	SD	0	0	-	SD
	Soft Tissue	2	2	1		2	2	1	
13B	Skeleton	0	0	-	SD	0	0	-	SD
	Soft Tissue	2	2	1		2	2	1	
14	Skeleton	24	30	1.25	PD	24	18	0.75	SD
	Soft Tissue	1	1	1		1	1	1	
15	Skeleton	9	14	1.5	PD	19	26	1.3	PD
	Soft Tissue	0	0			0	0	-	

Patient 2 had a normal FDG PET/CT pre and post ^{131}I-mIBG therapy.

Table 6.7 Skeletal scores only. Patients 9, 11, 12, 13A and 13B had soft tissue disease only (shaded)

PATIENT	mIBG Score Pre	mIBG Score Post	mIBG Relative Score	mIBG Response	FDG Score Pre	FDG Score Post	FDG Relative Score	FDG Response	PERCIST
1	7	0	0	CR	3	0	0	CR	CMR
2	4	0	0	CR	0	0	-	-	-
3	11	5	0.45	PR	11	10	0.9	SD	PMR
4A	16	11	0.7	MR	12	0	0	CR	CMR
4B	11	3	0.3	PR	0	0	-	-	-
5	54	47	0.9	SD	30	18	0.6	MR	PMR
6	13	4	0.3	PR	32	0	0	CR	CMR
7	26	4	0.15	PR	9	0	0	CR	CMR
8	1	0	0	CR	11	3	0.3	PR	PMR
9	0	0	-	-	0	0	-	SD	-
10	8	8	1	SD	16	0	0	CR	CMR
11	0	0	-	-	0	0	-	-	-
12	0	0	-	-	0	0	-	-	-
13A	0	0	-	-	0	0	-	-	-
13B	0	0	-	-	0	0	-	-	-
14	24	30	1.25	PD	24	18	0.75	SD	SMD
15	9	14	1.5	PD	19	26	1.3	PD	PD

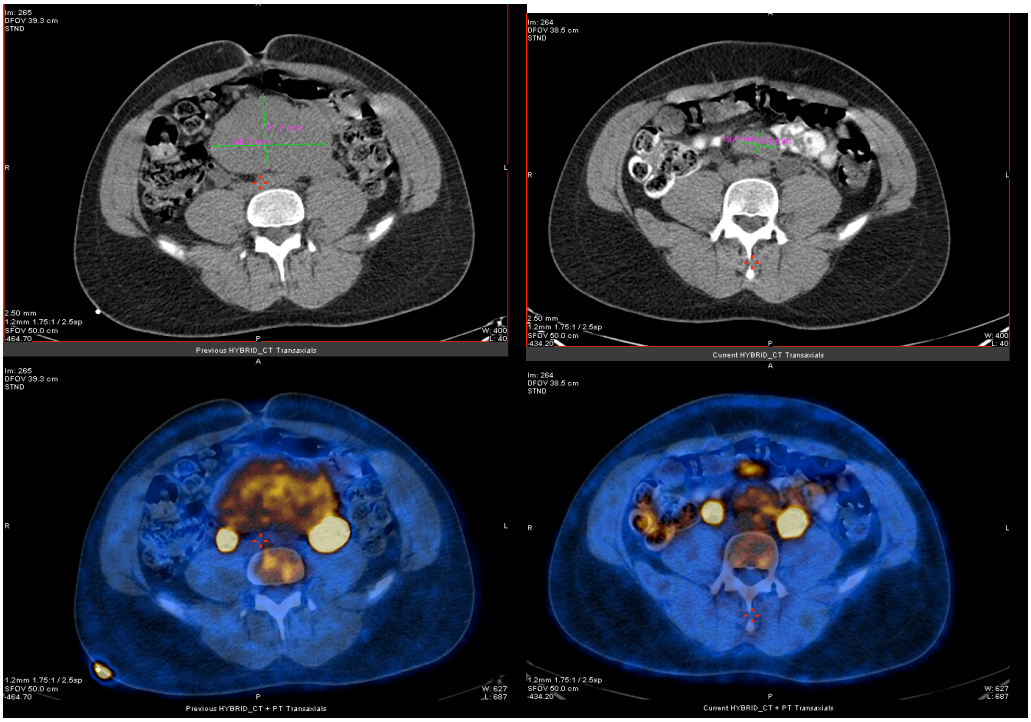
^{18}F-FDG PET/CT gave additional information to ^{123}I-mIBG scintigraphy in 5 out of 12 cases with skeletal disease. In 2 patients the ^{123}I-mIBG scan had showed stable disease but the ^{18}F-FDG PET/CT showed a partial or complete metabolic response by PERCIST. In 2 patients the ^{123}I-mIBG scan had showed a partial response to therapy but the ^{18}F-FDG PET/CT showed a complete metabolic response in both cases by PERCIST. In the case of patient 4A, the ^{123}I-mIBG scan only showed a minor response but the ^{18}F-FDG PET/CT showed a complete response by semi-quantitative scoring and a complete metabolic response by PERCIST. Conversely, patient 8 had a complete response on the ^{123}I-mIBG scan but only a partial response on the ^{18}F-FDG PET/CT by semi-quantitative and PERCIST scoring.

Table 6.8 Patients with soft tissue disease only prior to therapy with ^{131}I-mIBG.

PATIENT		mIBG Score Pre	mIBG Score Post	mIBG Relative Score	mIBG Response	FDG Score Pre	FDG Score Post	FDG Relative Score	FDG Response	PERCIST
9	Soft Tissue	4	4	1	SD	4	4	1	SD	CMR
11	Soft Tissue	2	2	1	SD	2	2	1	SD	SMD
12	Soft Tissue	1	1	1	SD	3	3	1	SD	PMR
13A	Soft Tissue	2	2	1	SD	2	2	1	SD	PMR
13B	Soft Tissue	2	2	1	SD	2	2	1	SD	SMD

For patients with soft tissue only disease, with the semi-quantitative scoring on ^{123}I-mIBG and ^{18}F-FDG PET/CT they all had stable soft tissue disease following treatment. In 3 instances, additional information was gained from the ^{18}F-FDG PET/CT as there was a metabolic response by PERCIST criteria on ^{18}F-FDG PET/CT indicting a response to therapy.

Figure 6.2A Patient 12, an 18 year old male, with Stage M (LN only metastases) refractory neuroblastoma treated with high-administered activity ^{131}I-mIBG and topotecan with peripheral blood stem cell support. This patient had soft tissue disease within the abdomen and pelvis only. As the semi-quantitative scoring only took into account a single or multiple soft tissue lesion for the score the disease was stable on ^{18}F-FDG PET and ^{123}I-mIBG imaging. There has been obvious regression of the mass on the CT component as well as a partial metabolic response by PERCIST criteria with the SUV_{max} reducing > 30% post treatment.



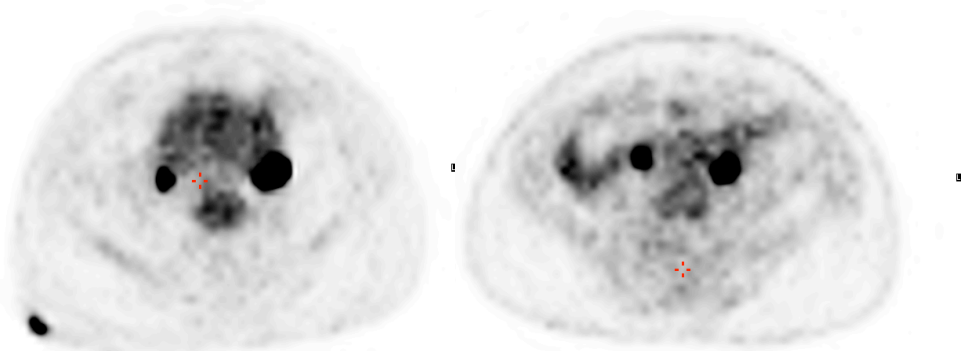


Figure 6.2B ^{123}I-mIBG planar scintigraphy (A and C) and ^{18}F-FDG PET MIP images (B and D) pre and post therapy with ^{131}I-mIBG for patient 6. In image D there is an artifact due to uptake of the tracer in the central venous access line. A partial response to ^{131}I-mIBG therapy was seen with the SIOPEN skeletal score changing from 13 to 4 on ^{123}I-mIBG planar scintigraphy but a complete response on ^{18}F-FDG PET/CT with SIOPEN score form 32 to 0 and a stable soft tissue component.

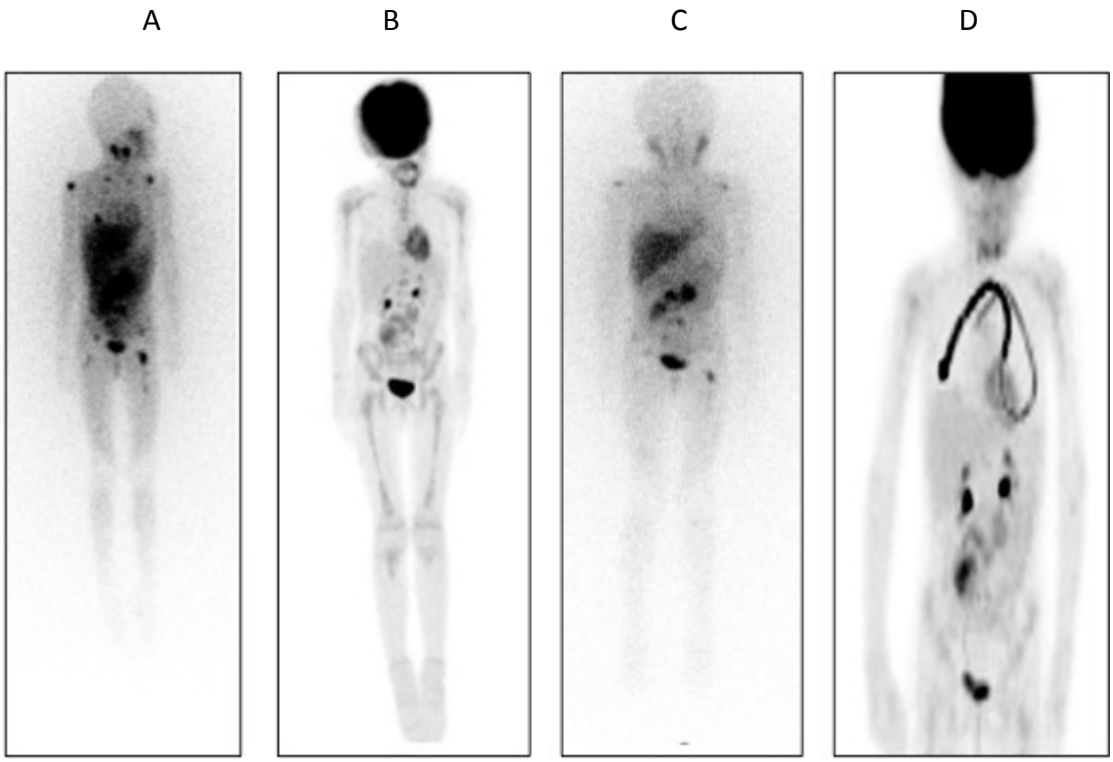


Figure 6.2C ^{123}I-mIBG planar scintigraphy (A and C) and ^{18}F-FDG MIP images (B and D) for patient 9 pre and post ^{131}I-mIBG therapy. There is significant uptake within brown fat seen in the ^{18}F-FDG MIP images. There were no skeletal metastases but a soft tissue mass in the left para-aortic, external and iliac chain and left inguinal none. The soft tissue masses were stable on semi-quantitative scoring of ^{123}I-mIBg and ^{18}F-FDG PET but had a complete metabolic response by PERCIST criteria with a SUV_{max} changing from 3.1 to 0 and 2 to 0.

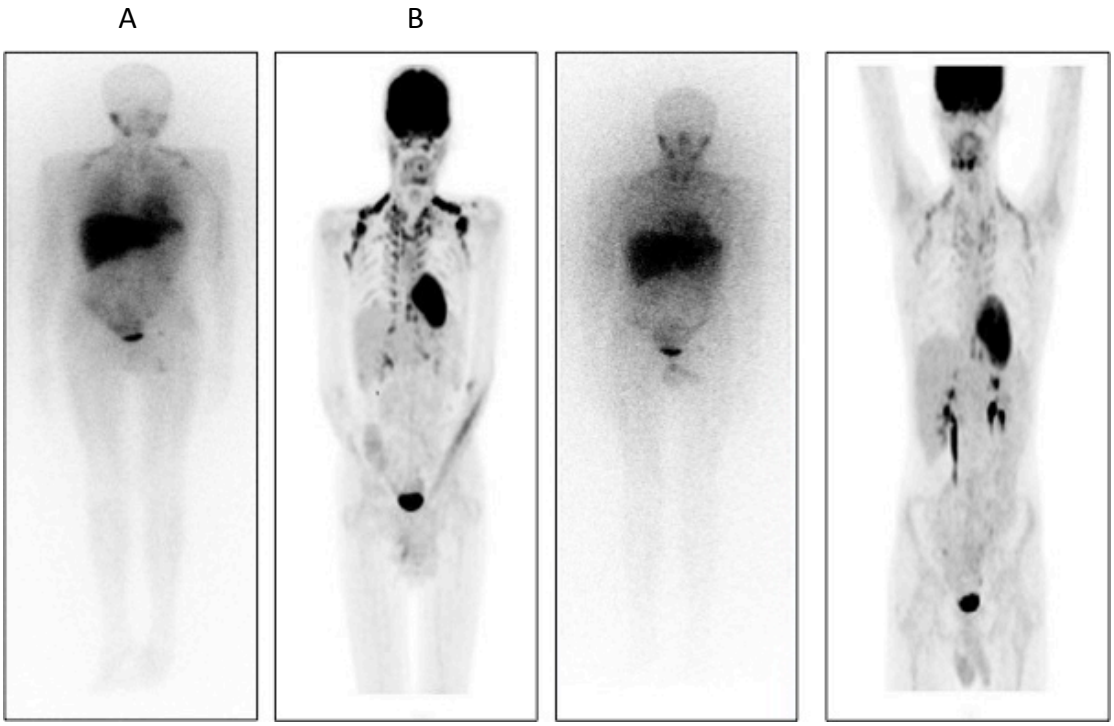
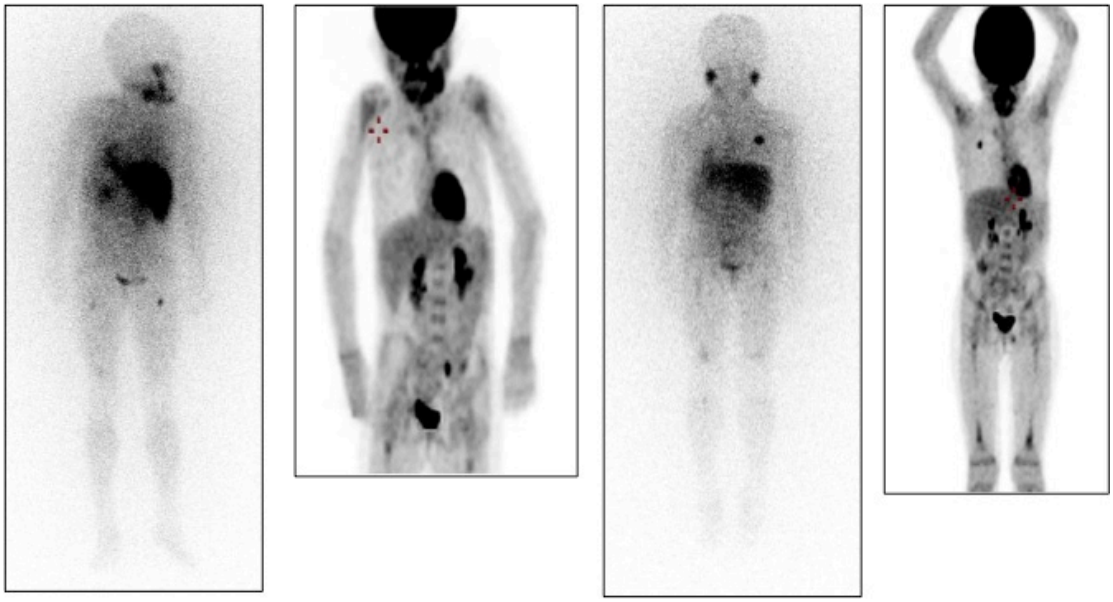


Figure 6.2D ^{123}I-mIBG planar scintigraphy and ^{18}F-FDG PET MIP images for Patient 15 pre and post ^{131}I-mIBG therapy. This patient had progressive disease on both imaging modalities with SIOPEN score for ^{123}I-mIBG increasing from 9 to 14 and increasing on ^{18}F-FDG PET from 19 to 26.



6.4 Discussion

^{123}I-mIBG imaging is the current gold-standard modality for imaging metastatic neuroblastoma. In a recent meta-analysis, ^{123}I-mIBG was found to have a sensitivity of 97% (95% CI, 95%-99%) for the detection of neuroblastoma although there was insufficient data to estimate specificity (*Jacobson 2010*).

There are limited prior reports on the use of ^{18}F-FDG PET in neuroblastoma and this study looked to examine whether imaging with ^{18}F-FDG PET/CT gave additional information to ^{123}I-mIBG scintigraphy in neuroblastoma patients treated with ^{131}I-mIBG molecular radiotherapy.

The first report of ^{18}F-FDG PET in neuroblastoma was in by Shulkin *et al*. in 1996. They compared ^{131}I-mIBG and ^{18}F-FDG PET in seventeen patients with neuroblastoma at various stages of disease and time points. Seven patients were examined at diagnosis prior to systemic therapy and ten patients were assessed at the time of suspected residual or recurrent disease. In all patients assessed prior to systemic therapy, the primary tumour was easily visualized with FDG PET. Overall, mIBG imaging was superior to FDG PET in 3 patients, FDG PET was considered to be superior to mIBG in 2 patients and the scans were found to be equivalent in 2 patients. In the thirteen instances where the imaging techniques were compared in the recurrent or residual disease setting, FDG PET was found to be superior in just

two instances, in one instance the images were similar and in 10 the mIBG imaging was found to be superior. Overall, they found that the tumour uptake of FDG to be lower after patients had received therapy than before. The SUV before therapy was greater than 2 but was less than 2 in those examined after systemic therapy or radiation. The other problem highlighted in this early study was the accumulation of FDG in the bone marrow whether or not it was infiltrated with neuroblastoma. They suggested that the main use of ^{18}F-FDG PET is likely to be in those neuroblastoma's that are mIBG negative (*Shulkin 1996*).

Sharp *et al.* performed a retrospective review comparing ^{123}I-mIBG and ^{18}F-FDG PET scans at the time of diagnosis. A total of 113 ^{123}I-mIBG and ^{18}F-FDG PET paired scans in 60 neuroblastoma patients were analysed. For the 10 patients with 13 paired scans in stage 1 or 2 neuroblastoma, ^{18}F-FDG PET was able to detect more primary tumour or local or regional metastases in 9 out of the 13 scans, the two imaging modalities were equal in 1 scan and 3 scans were normal. For the 10 stage 3 patients, ^{123}I-mIBG detected more extensive primary disease or regional metastases in 5 of 15 scans, and ^{18}F-FDG PET was able to do this in 4 of the 15 scans. For the 40 stage 4 neuroblastoma patients there were 85 paired scans evaluated. ^{123}I-mIBG depicted more neuroblastoma than ^{18}F-FDG PET in 44 of 85 scans and ^{18}F-FDG PET depicted more disease in 11 out of 85 scans. They concluded that ^{123}I-mIBG was better for the evaluation of stage 4 disease as it can detect more bone or bone marrow disease *(Sharp 2009*).

Melzer *et al.* performed ^{18}F-FDG PET scans in a cohort of neuroblastoma patients with ^{123}I-mIBG negative scans, discrepancy on ^{123}I-mIBG and morphological imaging or in patients whose clinical findings were inconsistent with initial imaging. There were 23, paired ^{123}I-mIBG and ^{18}F-FDG PET scans in 19 paediatric patients. Out of a total of 58 suspicious lesions the sensitivity of ^{123}I-mIBG was 50% compared to 78% for ^{18}F-FDG PET. The specificity was 75% for ^{123}I-mIBG and 92% for ^{18}F-FDG PET. They concluded that ^{18}F-FDG PET could be recommended if there were inconclusive findings or discrepancies on the ^{123}I-mIBG imaging (*Melzer 2011*).

Papathanasiou et al compared ^{123}I-mIBG and ^{18}F-FDG PET/CT scans in 28 patients with relapsed or refractory neuroblastoma prior to treatment with high-dose ^{131}I-mIBG therapy. ^{123}I-mIBG was superior to ^{18}F-FDG PET/CT in mapping disease extent for these patients. However, in the 14% of patients who had more disease on ^{18}F-FDG PET/CT than ^{123}I-mIBG, the outcome was statistically worse (*Papathanasiou 2011)*.

One other group has explored the role of ^{18}F-FDG PET/CT in patients treated with ^{131}I-mIBG molecular radiotherapy (*Taggart 2009*). The study included patients who had poorly responsive or progressive high-risk neuroblastoma. Patients were treated within a dose escalation study of ^{131}I-mIBG given on days 0 and 14 with autologous peripheral blood stem cell re-infusion on day 28. 14 patients had both ^{18}F-FDG PET and ^{123}I-mIBG scans performed but only 9 patients had paired, concomitant ^{18}F-FDG PET and ^{123}I-mIBG scans pre and post treatment with ^{131}I-mIBG. The imaging was performed in multiple treatment centres and the authors state that there was

variation in the body views obtained. For both soft tissue and bone lesions combined, they found ^{123}I-mIBG imaging to be significantly more sensitive than ^{18}F-FDG PET both pre and post ^{131}I-mIBG therapy. This remained the case when bone lesions only were examined. There was a suggestion, although not statistically significant, that ^{18}F-FDG PET may be more sensitive than ^{123}I-mIBG for detecting soft tissue lesions. The ^{18}F-FDG PET scans became completely negative post treatment more often than ^{123}I-mIBG scans. The analysis on response, comparing the two different imaging modalities, looked only at the number of lesions on the two scans and did not include metabolic response information from changes in SUV_{max} scores from the ^{18}F-FDG PET scans as we performed in our study. Within the study, discussed above by *Taggart et al.*, there were some patients who had had ^{18}F-FDG PET scans performed at day 13 and day 56 post ^{131}I-mIBG therapy. Two of the five patients showed a response at day 56 which was not seen on the day 13 ^{18}F-FDG PET scan (*Taggart 2009*).

For the cohort of patients discussed in this chapter, the distribution of disease on the two different imaging modalities was often discordant before the start of treatment. The distribution of skeletal disease was often highly variable between the two imaging modalities. For those patients with soft tissue disease only, the disease was seen on both scans in the same areas and therefore direct comparison was much easier.

The SUV_{max} scores on ^{18}F-FDG PET/CT in the bones were low and there is no way of knowing whether they were all disease related, especially if they did not correspond

to areas of disease on the ^{123}I-mIBG scans. All of the patients in this study had mIBG positive disease as they were pre-selected patients who were going to undertake mIBG therapy. In 6 patients, the initial ^{18}F-FDG PET/CT semi-quantitative score was higher than on the ^{123}I-mIBG scan.

For those four patients with soft tissue only disease, the extent of disease was identical between the ^{123}I-mIBG and ^{18}F-FDG PET/CT scans on semi-quantitative scoring, showing stable disease in all cases. The ^{18}F-FDG PET/CT PERCIST score was able to give additional response information and demonstrated a complete metabolic response or partial metabolic response in three patients. The number of patients examined in this study was too few to establish whether the metabolic response seen on ^{18}F-FDG PET/CT translates to improved outcome for those patients.

The optimal time for response assessment imaging following ^{131}I-mIBG is not clearly defined but should be performed at least 6 weeks after therapy. The majority of the post treatment scans in this series were performed within 3 months after therapy but there were some that occurred considerably later than this for logistical reasons. For those patients who had a metabolic response on ^{18}F-FDG PET/CT compared to stable disease or partial response, it is possible that metabolic response is seen earlier on FDG PET as the glucose uptake mechanism is the first to be turned off. For one of the patients who had a complete response, the scan wasn't performed until 5 months after treatment and we don't know if the same response would have been seen at an earlier time point.

Limitations of ^{18}F-FDG PET in the setting of refractory and relapsed disease include low-grade uptake in bones, which may have been a result of bone marrow hyperplasia in patients treated with recent cytotoxic chemotherapy or the use of G-CSF. ^{18}F-FDG PET/CT may be of limited value for the evaluation of bone marrow involvement due to the mild accumulation of FDG in normal bone marrow. The principal drawback of ^{18}F-FDG PET/CT is its low accuracy, during staging and restaging, in detecting NB localization in bone and bone marrow, which are the most frequent sites of disease progression (*Kushner 2001, Taggart 2009*, *Papathanasiou 2011)*.

None of the patients in this series were known to have brain metastases. mIBG is not thought to cross the blood-brain barrier and has limited ability for detecting intracranial disease. The high uptake of FDG within brain tissue also limits the use of ^{18}F-FDG PET/CT for the detection of brain and skull metastases. If brain metastases were suspected a MRI would still be required (see chapter 1).

One of the difficulties when examining a new functional imaging modality is knowing how best to score and interpret the scans. For mIBG scans, a semi-quantitative scoring system is recommended (see chapter 1). The 2 most commonly used semi-quantitative scoring systems, SIOPEN and Curie, do not take into account soft tissue disease and only score the extent of disease in the skeleton. Their main evaluation has been in the assessment of response to primary treatment rather than in the relapsed or refractory setting. Metastatic soft tissue disease is very unusual at the

initial presentation of metastatic neuroblastoma. The response on mIBG scans post induction chemotherapy is what determines progressive to high-dose myeloablative therapy and patients will have subsequent surgical resection of their primary tumour as part of their multi-modality treatment. Soft tissue metastases are much more common in relapsed disease and therefore our scoring of disease extent incorporated a measure of soft-tissue disease as well as skeletal disease.

Introducing new functional imaging modalities for assessment in neuroblastoma is complicated. Most published reports do initially include small numbers of patients from single institutional studies. The scans need to be performed in addition to standard imaging modalities and this has implications in terms of time, expense, radiation exposure and for smaller children an extra general anaesthetic.

The debate remains as to when and in which patients ^{18}F-FDG PET/CT may be most useful. Response on ^{18}F-FDG PET/CT indicating a biological response to therapy may be of clinical value in early response assessment to induction therapy or the assessment of response to novel agents in early phase clinical trials. With current high-risk neuroblastoma regimes, patients receiving induction chemotherapy are assessed at the end of therapy with a ^{123}I-mIBG scan. Approximately one third of patients will not respond to induction therapy. If these patients could be identified as 'poor responders' earlier on in their treatment course by an ^{18}F-F-FDG PET/CT then they could be offered alternative treatment strategies.

In a small study, Chawla et al evaluated the role of ^{18}F-FDG PET/CT in staging and early treatment response to chemotherapy in 17 children with neuroblastoma. They concluded that it was a good modality for response assessment in patients with moderate or high FDG uptake on the baseline scans but that it was of no benefit in patients with low baseline FDG uptake (*Chawla 2010*).

^{18}F-FDG PET/CT gives supplementary information to ^{123}I-mIBG scintigraphy in the response assessment of relapsed or refractory neuroblastoma in some cases. Because of the discrepancy in mapping skeletal disease in this cohort of heavily pre-treated patients, the main benefit for ^{18}F-FDG PET/CT appeared to be in giving additional information on metabolic response in patients with soft tissue disease only, who otherwise may have been scored as stable disease only. The clinical significance of this is uncertain and requires further evaluation and correlation with outcome in a larger cohort of patients.

Chapter 7

Evaluation of Intensity Modulated Arc Therapy for abdominal neuroblastoma

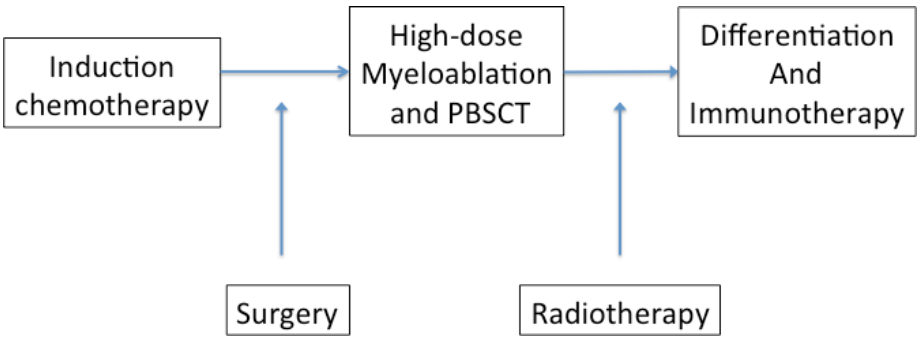
7.1 Introduction

External beam radiotherapy forms part of the multi-modality treatment of high-risk neuroblastoma. It is given, along with surgical resection of the primary tumour, with the aim of reducing the risk of local recurrence (see chapter 1). New treatments for high-risk neuroblastoma will hopefully improve the outcome, but so may improvements in the delivery of existing treatments, including radiotherapy.

High-risk neuroblastoma trial protocols from around the world are similar in their multi-modality approach. Patients within the UK are currently treated on the International Society of Paediatric Oncology (Europe) Neuroblastoma (SIOPEN) Group's high-risk neuroblastoma protocol – SIOP HR-NBL1.

Figure 7.1

Illustration of where the different components of multi-modality therapy fit into the SIOP HR-NBL1 trial.



Radiotherapy is given after myeloablative chemotherapy and peripheral blood stem cell rescue and prior to the patient commencing differentiating therapy or immunotherapy. The reasons for the timing of radiotherapy are a) because radiation effects on bone marrow or other organs (gastrointestinal system, liver, kidneys) could potentially interfere with chemotherapy delivery and b) in theory a large primary tumor in-situ is more likely to be hypoxic and therefore relatively radioresistant compared to smaller tumors or microscopic residual disease. The interval after BUMEL myeloablative therapy / stem cell rescue and radiotherapy must be greater than 60 days in the SIOP HR-NBL1 study, due to the risk of busulfan enhancing the toxicity of radiotherapy (*Seddon 2005*). There is also a negative association between isoretinoin (used for differentiating therapy) and radiotherapy and therefore 13-cis-retinoic acid is not commenced until after the completion of radiotherapy.

Within the SIOP HR-NBL1 trial, radiotherapy is given to all patients to the initial tumour site, independent of the extent of surgical resection. Similarly, the prescribed dose is standardised, regardless of the presence of any macroscopic residual disease, the tumour biology or the age of the patient. The radiotherapy planning is based on the post induction chemotherapy, pre-operative gross tumour volume (GTV) as shown by imaging. Post-operative notes and pathological reports are also taken into account when defining the GTV. The Clinical Target Volume (CTV) is the sum of the pre-operative GTV, areas of persistent adjacent lymph node enlargement after induction therapy and all areas of microscopic spread on the surgical and pathological reports. A margin is added for uncertainties in positioning

to create the final Planning Target Volume (PTV). Total margins are therefore typically 2cm in each direction.

External beam radiotherapy for high-risk neuroblastoma within Europe has been traditionally delivered with an anterior and posterior parallel-opposed pair of fields. Customised blocks or multi leaf collimation have been used to reduce unnecessary irradiation of normal tissues. The dose prescribed is 21Gy in 14 fractions, 1.5Gy per fraction, treating 5 fractions a week.

Delivery of the full protocol dose with the conventional radiotherapy technique is frequently limited by the proximity of the kidneys and liver and this compromise on target volume coverage could impact on local control and ultimately outcome for patients with high-risk neuroblastoma.

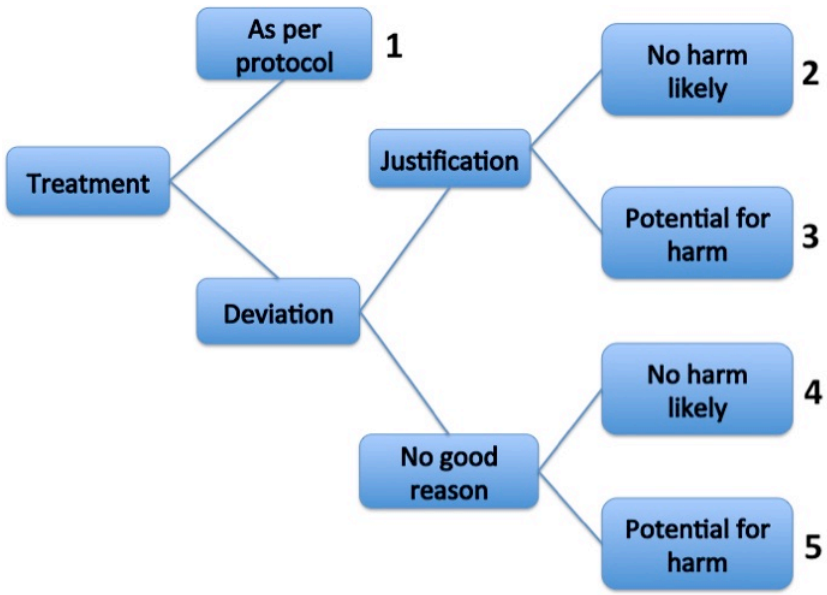
The dose received by critical organs at risk such as the kidneys and liver are especially important in this paediatric population who have received intensive multi-modality therapy including high dose myeloablative chemotherapy and nephrotoxic agents. Within the paediatric population there are additional long-term factors to be taken into account when using radiotherapy such as the effect on growing tissues and the risk of developing a second malignancy.

Within University College London Hospital NHS Foundation Trust we audited 41 patients who had been treated with conventional radiotherapy between 2006 and 2010 and found that 20 (49%) of them had required a modification in dose or PTV

coverage to stay within organ at risk dose constraints with the conventional radiotherapy technique. A compromise on target volume coverage or reduction in dose, or both, could have an impact on local control.

Within Europe, I have been part of the SIOPEN high-risk neuroblastoma radiotherapy quality assurance programme (*Gaze 2010*). The SIOPEN trial electronic central database has the ability for individual treatment centres to remotely upload diagnostic imaging and radiotherapy planning data for central review. A quality scoring system was devised to allocate cases to one of five quality assurance scores as shown below.

Figure 7.2 Quality assurance flow chart



Central review of diagnostic and radiotherapy planning data sets was retrospectively performed by a team of international paediatric radiologists and clinical oncologists. Quality assurance review of radiotherapy delivered in 100 patients in the trial showed only a 48% compliance with the radiotherapy protocol recommendations. In 29% of the patients there was a justifiable reason for the deviation with no likely long-term consequences, most commonly to spare organs at risk. In 5% of cases there were justifiable reasons for the deviation but with likely long-term consequences. In 1% there was a non-justifiable deviation but without likely long-term effects. In 17%, the protocol was deviated for unjustifiable reasons with a risk of adverse events as a result. Results of this work were published in the International Journal of Radiation Oncology Biology and Physics. (*Gaze 2013*). On going work within the SIOPEN radiotherapy group will examine whether radiotherapy protocol deviations have impacted on local recurrence or outcome in a larger cohort of patients.

The past decade has seen significant developments in radiotherapy techniques. Intensity Modulated Radiotherapy (IMRT) offers a high degree of conformity to the target volume and a reduction in dose to nearby organs at risk and normal tissue. IMRT involves the production of non-uniform beam intensity across a field using advanced planning, verification and delivery techniques.

One form of IMRT is Intensity Modulated Arc Therapy (IMAT). IMAT was originally proposed by Yu in 1995 and there are now several commercially available forms of IMAT. RapidArc^{TM} (Varian Medical Systems) is one such IMAT technique which uses

single or multiple arc gantry rotations with variable dose rate, gantry speed and MLC aperture to produce highly conformal dose distributions. RadpidArc™ is the technique available within my research institution and therefore the IMAT technique discussed here.

IMRT techniques can often result in a larger volume of normal tissue receiving a low dose of radiation. The long-term effects, especially carcinogenesis, of this so called 'low-dose bath effect', has not been quantified in the paediatric setting and remains controversial. It is essential therefore that these techniques, which have theoretical advantages for tumour volume coverage, are scientifically evaluated before their routine adoption into paediatric radiotherapy practise.

A retrospective planning study was therefore performed to compare RapidArc™ IMAT with the current standard practise in a cohort of high-risk neuroblastoma patients who had already been treated with a conventionally planned radiotherapy technique.

The aim of the planning study was to establish whether IMAT enabled

1. a greater proportion of patients able to receive the full protocol dose within normal dose constraints
2. improved tumour volume coverage and reduced organ at risk doses in patients who had been able to receive the full protocol dose.

Three cases where the IMAT technique have been used clinically are also presented at the end of the planning study.

7.2 Patients and Methods

Patient selection

Twenty children who had received conventional radiotherapy between 2006 and 2010 were identified. All patients had been treated at University College London Hospitals NHS Foundation Trust.

The median age was 3 years (range 1-9 years). There were 11 boys and 9 girls. The INSS stage was 4 in 12 patients and INSS stage 3 in 8 patients. Eleven of the patients had well-lateralised tumours (6 left-sided, 5 right-sided) and 9 patients had midline tumours.

Of the 20 children, 10 were what we termed '*protocol compliant'* and had received the full prescribed and intended protocol dose of 21Gy to the PTV in a single phase with conventional radiotherapy planning and within the organ dose constraints.

The other 10 patients were termed '*protocol non-compliant'.* In this group it had not been possible to deliver the full protocol dose of 21Gy to the PTV within the tolerance doses of organs at risk. These *protocol non-compliant* patients had therefore been treated with either a reduced dose or a reduce coverage of the PTV.

Table 7.1

Table showing the radiotherapy doses received by the 10 patients in the protocol non-complaint group. There was either compromise in dose, tumour volume coverage or both. The phase 2 did not take the *full* PTV to 21Gy.

Patient Number	Phase I dose (Gy)	Phase II dose (Gy)	Total dose (Gy)
11	12	9	21
12	10.5	10.5	21
13	18	3	21
14	15	6	21
15	15	0	15
16	15	6	21
17	15	0	15
18	15	6	21
19	13.5	7.5	21
20	15	6	21

Volume Definition

All 20 patients had a Computed Tomography (CT) scan (GE light speed 16 slice) simulation with 2.5mm slices. The GTV was based on the extent of the primary tumour and abnormal adjacent lymph nodes – on post-induction chemotherapy, pre-operative imaging. This imaging was fused with the planning CT scan for target volume definition. There are potential geometric errors in this method but fusing the pre-operative imaging is used as a guide to target volume definition and planning is performed post-operatively when there are significant anatomical changes and location of organs post surgical resection of the primary tumour.

Once the GTV had been defined a 1.5 cm margin was added in 3 dimensions to form a CTV to cover areas of microscopic disease. The CTV was edited back in the areas of liver and kidney to a margin of 0.5cm unless there was evidence from imaging, surgery or pathology that there had been infiltration by the primary tumour. A further 0.5cm margin was added to the CTV to allow for uncertainties in positioning and organ motion to form a PTV.

Both kidneys and liver were contoured as organs at risk. The vertebrae at the level of the PTV were also contoured so that the dose homogeneity within theses irradiated vertebrae could be calculated. For all patients, there was significant overlap of the PTV and vertebra and therefore the contiguous vertebrae were included in the PTV for all the IMAT plans.

Conventional Planning

The conventional radiotherapy plans were optimised on Oncentra masterplan v3.2 (Nucleotron). All but one of the patients had been treated with a standard AP/PA technique. The other patient had been treated with a 3-field technique.

Nineteen patients had been treated with 6MV photons and one with 10MV photons. The upper and lower field borders for the conventional plans had been extended to the next inter-vertebral space to ensure the radiation effects on vertebral growth were symmetrical.

IMAT planning

The IMAT (RapidArc^{TM}) plans were optimised on Eclipse v8.6 (Varian Medical Systems). After the first few patients had been planned with the IMAT technique, we found superior coverage with 2 arcs compared to a single arc and therefore all subsequent plans were done using dual arcs. The first arc was applied clockwise from 179° to 181° with a 20° collimator angle (Varian IEC scale). The second arc was applied anti-clockwise form 181° to 179° with a 340° collimator angle.

Plan Optimisation

Dose distributions for the conventional treatments were achieved through iterative forward planning and in multiple phases if necessary, to respect normal tissue tolerance.

IMAT plans utilised the Eclipse Progressive Resolution Optimiser (PRO II) - an algorithm developed from that first proposed by Otto in 2008. The algorithm optimizes dose rate, gantry speed and MLC aperture with the convergence towards a final solution which improves through five resolution levels.

Dose volume objectives were added for target volumes, organs at risk and normal tissue in order to achieve the plan objectives (detailed in the plan comparison section).

Plan Dose Calculation and Normalisation

The conventional plans were calculated using the Oncentra Masterplan Pencil Beam Convolution Algorithm. Plans were normalised with 100% prescription to a point in accordance with ICRU report 62 (Prescribing, Recording and Reporting Photon Beam Therapy).

The IMAT plans were calculated using the Eclipse Anisotropic Analytical Algorithm (AAA). Plans were normalised with 100% prescription to the target volume mean dose in accordance with ICRU report 83 (Prescribing, recording and reporting photon-beam intensity-modulated radiotherapy).

It is noted that a different treatment planning algorithm has been used for both of the techniques and therefore a cohort of conventionally planned patients were re-planned with the AAA algorithm to see if there was a significant change in dose distribution. No significant difference was observed and therefore the dose volume statistics from the Oncentra Masterplan Pencil Beam Convolution Algorithm has been recorded for the conventionally treated patients.

Plan Comparison

The dosimetric parameters of the IMAT and conventionally plans were directly compared.

PTV

To asses the plan quality with respect to the PTV coverage we looked at:

1. The $D_{98\%}$ - the dose received by 98% of the PTV volume
2. The $D_{2\%}$ - the dose received by 2% of the PTV volume
3. The Conformity Index (CI) – the ratio of the volume covered by the 95% isodose to the volume of the PTV
4. The homogeneity Index (HI) – the ratio of $(D_{2\%} - D_{98\%}) / D_{50\%}$

   ($D_{50\%}$ = dose receive by 50% of the PTV volume)

These 4 criteria are those recommended by ICRU 83.

Organs at Risk

Kidneys

For lateralised tumours, where the ipsilateral kidney would receive 21Gy, the contralateral kidney dose limit was a $V_{12} \leq$ 10%. For midline tumours, a combined kidney $V_{12} \leq 35\%$ was used.

Liver

A V_{19} ≤100% and a V_{21} ≤ 25% were used as per the liver dose constraints used in the current SIOPEN high-risk protocol.

Vertebrae

A homogenous distribution of dose to the vertebrae in children receiving external beam radiotherapy is critical to future growth and the risk of deformities in spinal growth (*Hogeboom 2001, Paulino 2000*). Dose homogeneity to the vertebra was assessed by the dose received by 98% of the vertebrae and a homogeneity index for the vertebra [($D_{2\%}$ - $D_{98\%}$) / $D_{50\%}$].

Normal Tissue

To measure the volume of normal tissue receiving a low dose of radiation, the non-PTV integral dose (NPID) was calculated for both conventional and IMAT plans and then compared. The NPID was calculated by the product of the mean dose to the Boolean structure, external minus PTV, by its volume in cm^3 (equal for both plans).

Statistical Analysis

The data was summarised using median and range. The normality was assessed using histograms. The Wilcoxon rank-sum and Wilcoxon matched-pairs signed-rank tests were used to compare groups. To account for multiple testing, the results were considered significant if the p value was less the 0.01. The calculations were performed using Stata v11. (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, Tx: StataCorp LP).

7.3 Results

Location

In the *protocol compliant group,* nine tumours were lateralised and one was a midline tumour. In the protocol non-complaint group two were lateralised and eight were midline.

Tumour Volume

There was no significant difference in the tumour volume between the *protocol complaint* and *protocol non-complaint groups*.

Median PTV volume

Protocol compliant group	392cm^3 (range 149-851)
Protocol non-compliant group	458cm^3 (range 251-781)

P=0.5

Tumour Coverage

The PTV coverage was compared for all 20 patients between conventional and IMAT plans. The tumour coverage was improved with IMAT plans as shown by statistically significant improvements in $D_{98\%}$, CI and HI as shown below:

Table 7.2 shows the comparison of PTV coverage for the IMAT and conventional techniques for all 20 patients in the study.

Parameter	Conventional Median (Range)	IMAT Median (Range)	Wicoxon rank-sum
CI	1.75 (0.9-2.7)	1.1 (1-1.2)	P <0.001
HI	0.33 (0.07-1.01)	0.09 (0.05-0.48)	P <0.001
$D_{98\%}$	15 (0.8-20.3)	19.9 (12.2-20.5)	P <0.001
$D_{2\%}$	21.8 (15-22.4)	21.8 (21.5-22.5)	P=0.7

Vertebral doses

The homogeneity of dose to the vertebra was improved with the IMAT technique.

Median D98%

Conventional 17Gy (range 0.8-20.4Gy)

IMAT 20.5Gy (Range 19-20.7Gy)

$p < 0.001$

Median Homogeneity Index

Conventional 0.24 (0.07-0.97)

IMAT 0.06 (range 0.05 – 0.14)

$p < 0.001$

Protocol Compliant Group

There were nine patients in this group with lateralised tumours, and therefore we directly compared 21Gy delivered by the conventional and IMAT techniques to look at kidney and liver doses.

There was no significant difference between the conventional and IMAT plans for both the ipsilateral and contralateral V_{15}'s and ipsilateral mean kidney doses. There was an increase in the contralateral mean kidney dose with IMAT.

There was no significantly significant difference in the mean liver dose between the two plans but the V_{19} was significantly reduced with the IMAT technique.

For the three patients with right-sided tumours in this group there was a reduction in the mean liver dose (median of 10.7Gy versus 12.9Gy) and V_{19} (median 0.1% versus 0.5%) with IMAT. For left sided tumours (6 patients) the mean dose to the liver was increased with IMAT (median 7.2Gy versus 4.1Gy) but there was a reduction in the V_{19} (median of 0.03% versus 0.13%).

Table 7.3 shows the liver and kidney doses for the nine patients with lateralised tumours in the protocol compliant group comparing 21Gy given conventionally and with the IMAT technique.

Variable	Plan		Wilcoxon-rank sum
	Conventional	IMAT	
Kidneys			
Contralateral V_{15} (%) median (range)	0.02 (0-0.1)	0.02 (0-0.07)	p = 0.4
Ipsilateral V_{15} (%) median (range)	0.67 (0.5-1)	0.61 (0.3-1)	p = 0.04
Contralateral mean dose (Gy) median(range)	2.3 (1-4)	6.7 (5.5-7.4)	p = 0.008
Ipsilateral mean dose (Gy) median(range)	15.8 (12-22)	15 (11-21)	p = 0.2
Liver			
mean liver dose (Gy) median (range)	6.6 (1.3-18.7)	7.5 (2.5-15.3)	p = 0.8
V19 (%) median (range)	0.24 (0-0.85)	0.03 (0-0.32)	P = 0.009

Examples in the protocol compliant

Figure 7.3 Left-sided lateralised tumour

Figure 7.3A

Comparison of conventional (left) and IMAT (right) plans for a left-sided lateralised neuroblastoma in the *protocol-compliant group*.

PTV = red, Liver = green, ipsilateral (left) kidney = blue, contralateral (right) kidney= orange. The 95% isodose curve is shown in red colourwash illustrating the increased conformity of the high-dose region with the IMAT technique.

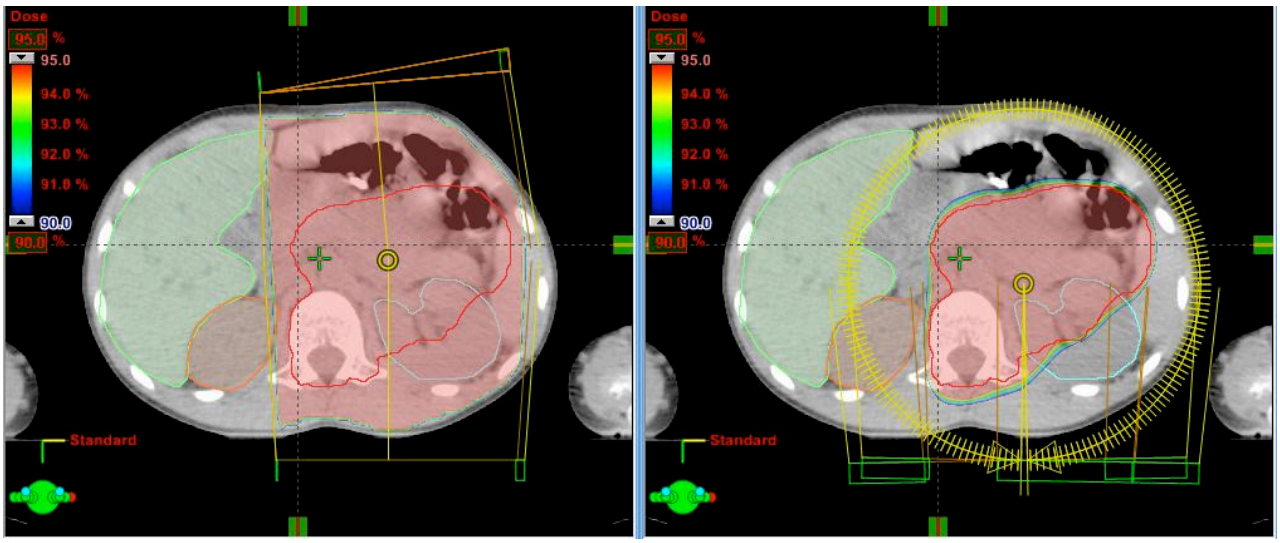


Figure 7.3B

Dose Volume Histogram (DVH) for the patient in Figure 7.3A, conventional (Δ) and IMAT (□) plans. The PTV coverage is comparable for the two plans. The IMAT plan allows some sparing of the ipsilateral kidney (blue) but with an increased dose to the contralateral kidney (orange).

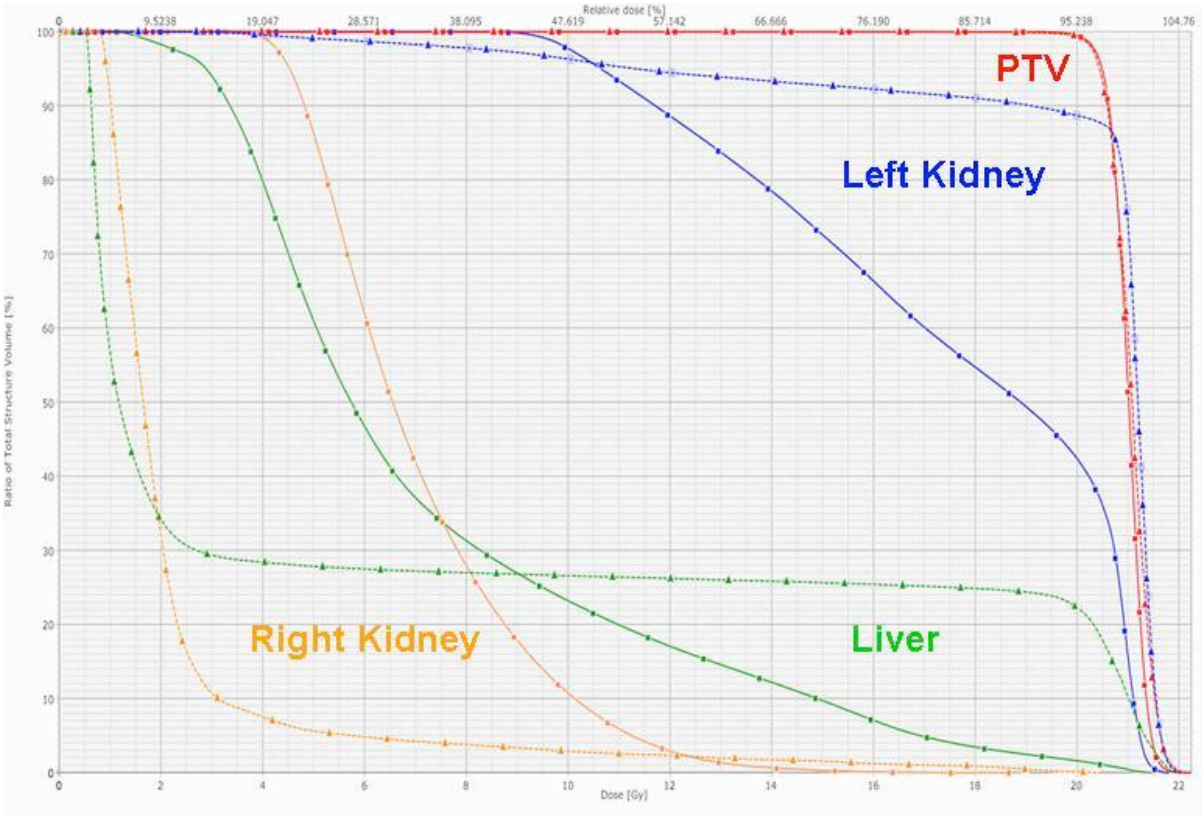


Figure 7.4 Right-sided lateralised tumour

Figure 7.4A

Comparison of conventional (left) and IMAT (right) plans for a right-sided abdominal neuroblastoma in the *protocol compliant group.* PTV = red, liver = green, ipsilateral (right) kidney = blue, contralteral (left) kidney = orange.

The 95% isodose curve is shown in colourwash illustrating the avoidance of the high dose volume to the liver with the IMAT technique.

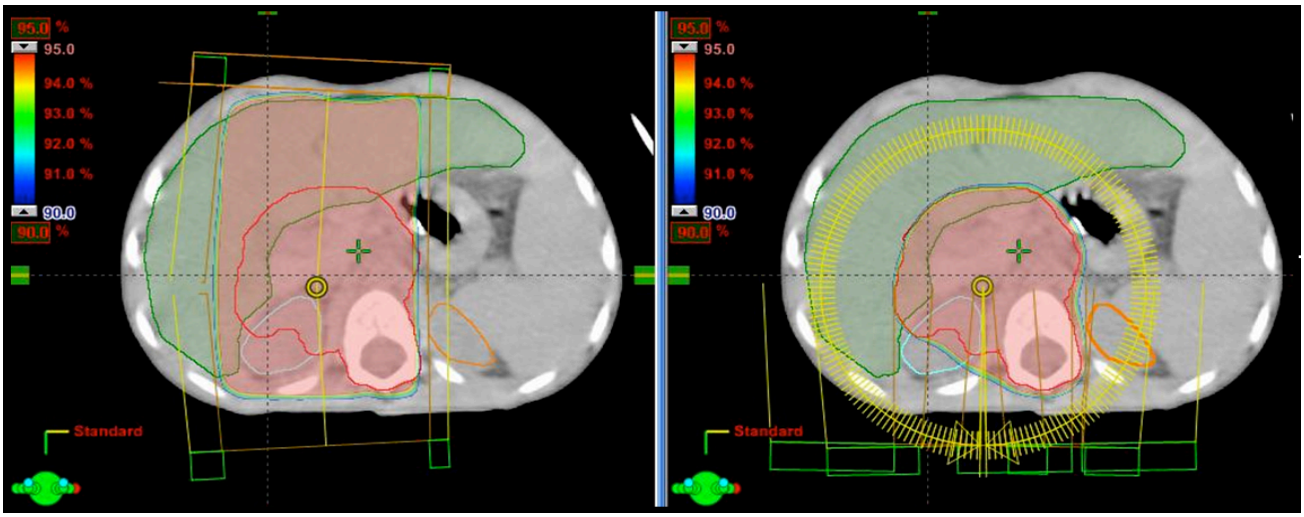
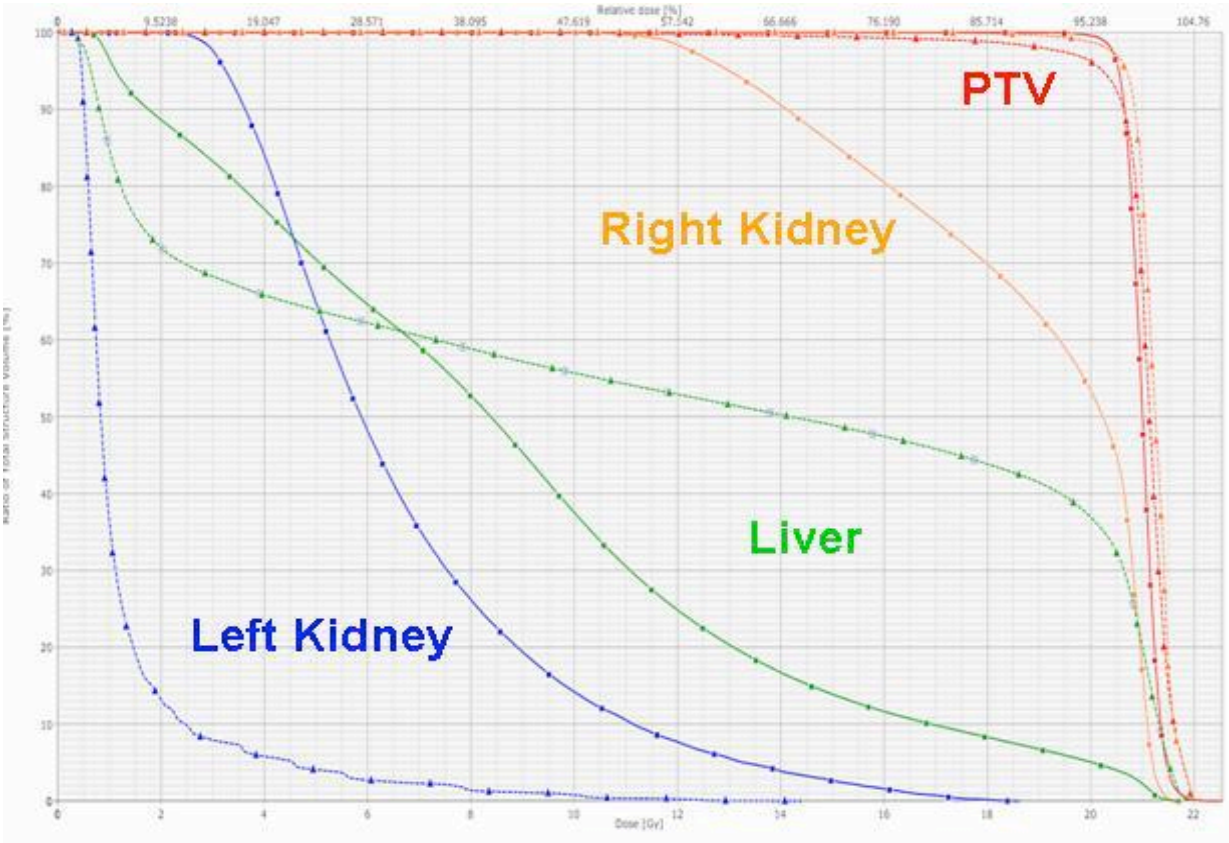


Figure 7.4B

The DVH for the patient in figure 7.4A. Conventional plan (Δ) and IMAT plans (□). The PTV coverage (red) between plans was comparable but the IMAT plan was able to spare some of the ipsilateral kidney (orange) and the liver (green) at the expense of an increased low dose to the contralateral kidney (blue).



Protocol Non-compliant Group

The majority of the tumours in this group were mid-line location and none of them had been able to receive 21Gy to the whole PTV with conventional AP/PA fields.

The IMAT technique could deliver the full protocol dose to the PTV within renal and hepatic dose constraints in 8 out of 10 cases.

The two cases where IMAT was unable to deliver the full protocol dose were both very large mid-line tumours. The plans were compared to see what percentage increase in dose could be achieved with the IMAT technique by comparing the $V_{19.95\%}$ (the volume receiving 95% of 21Gy). For one patient, the increase was from 64% to 89% and the other (in whom only 15Gy had been possible conventionally) form 0% to 94% with IMAT.

The two lateralised tumours in this group were both right–sided and the IMAT technique reduced the high dose region to the liver.

Example from the protocol non-compliant group

Figure 7.5 A mid-line neuroblastoma

Figure 7.5A

Comparison of conventional (left) and IMAT (right) plans in a patient in the protocol non-compliant group with a midline tumour (PTV in red). The colourwash shows the dose coverage between 15Gy and 21Gy, highlighting the under-coverage of the PTV in the conventional plan.

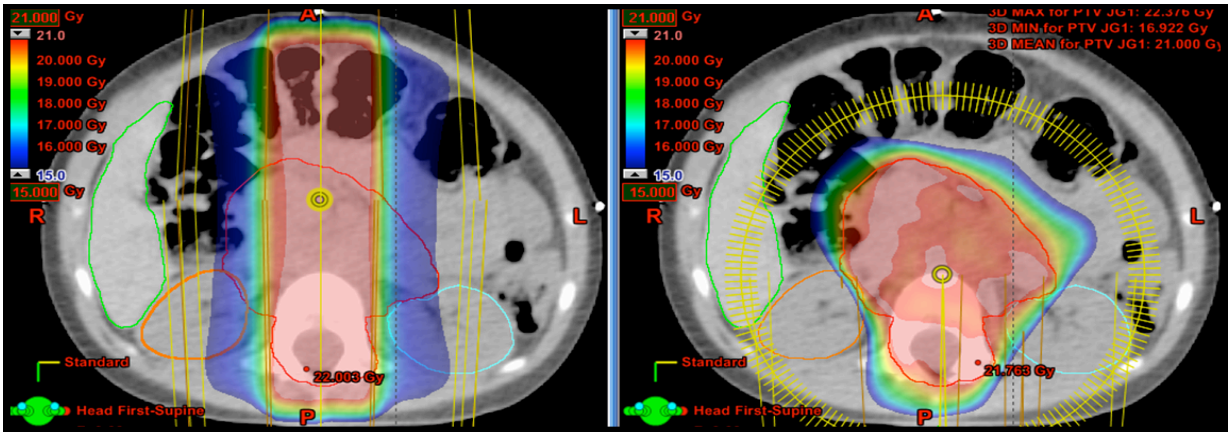
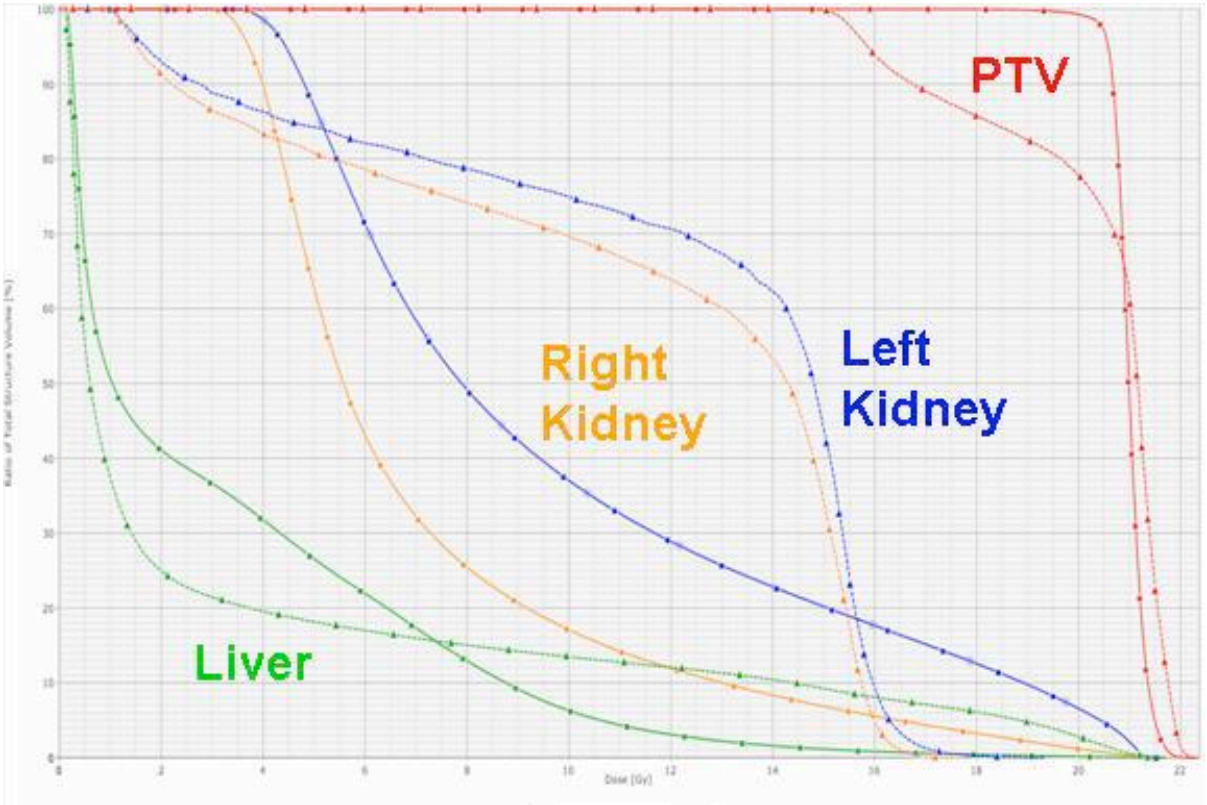


Figure 7.5 B

DVH for the patient in 7.5A, conventional (Δ) and IMAT (□) plans. The PTV coverage (red) is improved with the IMAT plans. The doses to both kidneys (right = orange, left=blue) and the liver (green) were all reduced with the IMAT technique.



Monitor Units

The monitor units (MU) were increased with the IMAT technique.

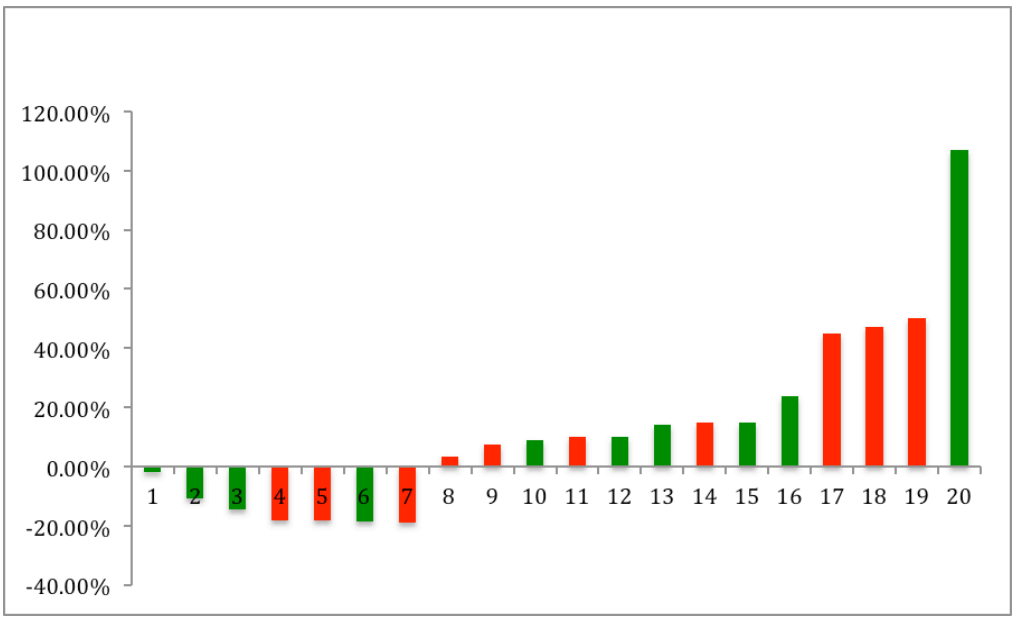
Median MU	
Conventional	162MU (range 131-334)
IMAT	328MU (range 261-432)

p <0.001

Non-PTV Integral dose

The NPID was variable between the patients with some having an increased NPID with the IMAT technique and some a reduced one. The protocol compliant patients are in green and the non-compliant in red.

Figure 7.6



Clinical Experience of IMAT in neuroblastoma

Following our experience in the planning study discussed above, as well as data from both our own institution and the European High-risk neuroblastoma trial, that many patients have a compromise on target volume coverage with conventional radiotherapy techniques we have recently taken the IMAT technique forward clinically in a select number of difficult cases.

The following three clinical cases illustrate current experience in using IMAT in clinical practise at UCLH for the treatment of high-risk neuroblastoma.

Clinical Case 1

A 4 year old girl with stage 4 neuroblastoma receiving treatment on the SIOP HR-NBL 1 trial. Planned to receive external beam radiotherapy to the primary abdominal tumour as part of the multi-modality treatment approach. Delivery of the full 21Gy in 14 fractions was unlikely to be possible in view of the large midline tumour in relation to the position of both kidneys. This patient was referred to us from another paediatric radiotherapy centre unable to offer Intensity Modulated Arc Therapy for paediatric patients.

The patient was 4 years old and with the expertise of our radiotherapy play specialists was able to have her 3 weeks of radiotherapy without a general anaesthetic. Treatment was tolerated well with no increased bowel or skin toxicity acutely.

Figure 7.7A illustrates the 2 phase AP/PA conventional plan generated showing that PTV coverage up to the dose of 21Gy in 14 fractions was not possible with conventional radiotherapy techniques. This plan would have involved a 2 phase technique with compromise on PTV coverage for the phase 2 to stay within renal tolerance. The Phase 1 (15Gy in 10 fractions) is shown in blue colourwash (15Gy) and the Phase 2 (6 Gy in 4 fractions) is shown in red colourwash.

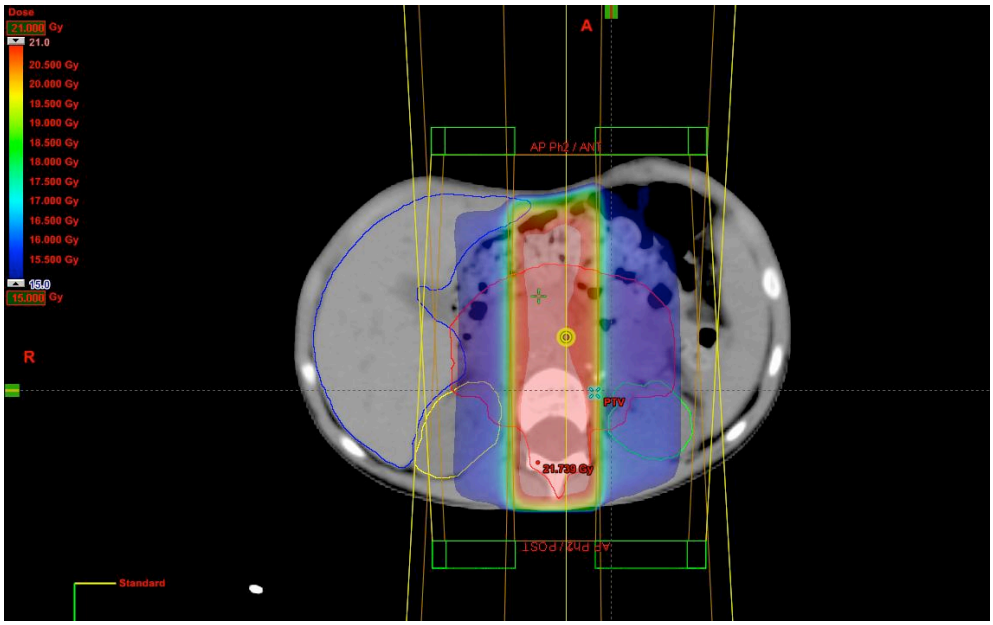
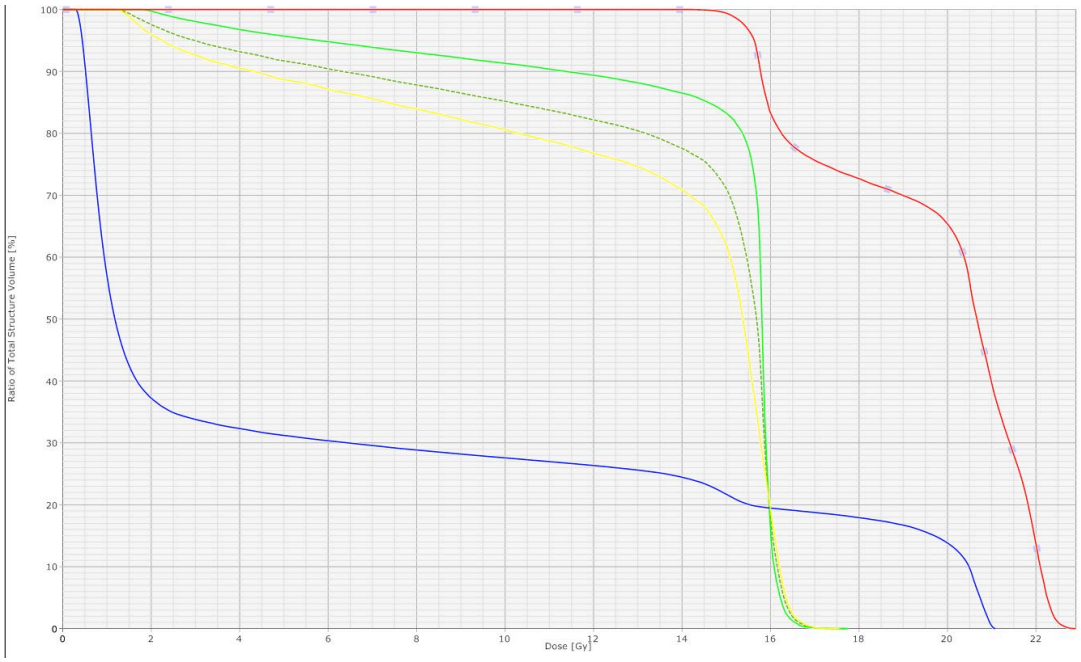


Figure 7.7B illustrates the DVH for the conventional plan showing under coverage of the PTV. Green = left kidney, blue = liver, yellow = right kidney, PTV = red , dotted green = combined kidneys.



An IMAT plan was generated which allowed full coverage of the PTV with the prescribed protocol dose of 21Gy.

Figure 7.7C illustrates the IMAT plan showing dull coverage of the PTV to 21Gy (red colourwash). As within the planning study the PTV was modified to include the vertebra to ensure homogenous dose to the vertebra and reduce the risk of long-term growth deformity in the spine.

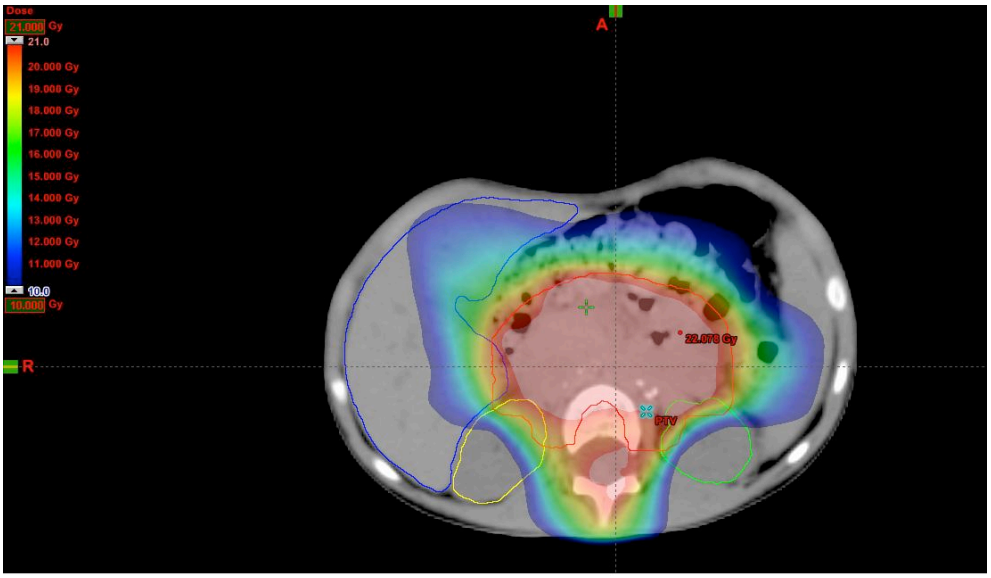
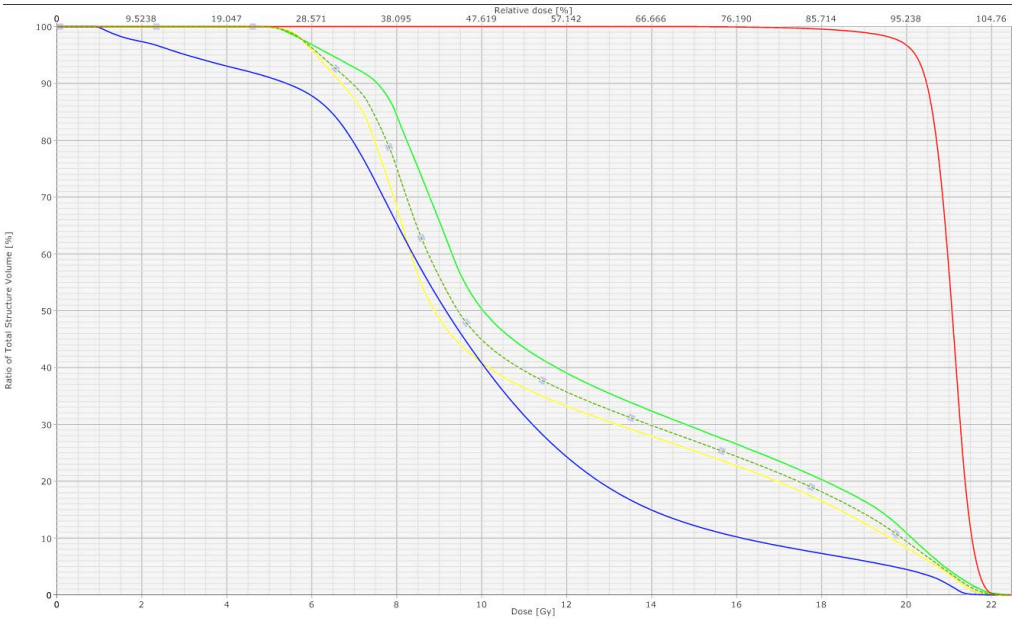


Figure 7.7D The DVH for the IMAT plan. Green = left kidney, blue = liver, yellow = right kidney, PTV = red , dotted green = combined kidneys. The IMAT plan enabled the full protocol dose of 21Gy to be delivered whilst staying within all organ at risk tolerance doses.



Clinical Case 2

A 14 year old girl with high-risk, stage M neuroblastoma being treated on the SIOPEN HR-NBL-1 trial referred for radiotherapy to the primary tumour in the abdomen as part of the multi-modality treatment of her high-risk neuroblastoma. At diagnosis there was a right suprarenal mass and metastases in the bone marrow as well as meningeal disease. Following surgical resection of the right suprarenal mass, there was residual disease within the abdomen. It was decided that she should have post-operative radiotherapy but at a higher dose of 36Gy in 20 fractions (1.8Gy/fraction). It would not have been possible to stay within normal tissue constraints with conventional radiotherapy and therefore the IMAT technique was adopted.

Figure 7.8A An axial image of the IMAT plan generated. The 36Gy isodose is shown in red colourwash and illustrates good coverage of the PTV with sparing of the left kidney.

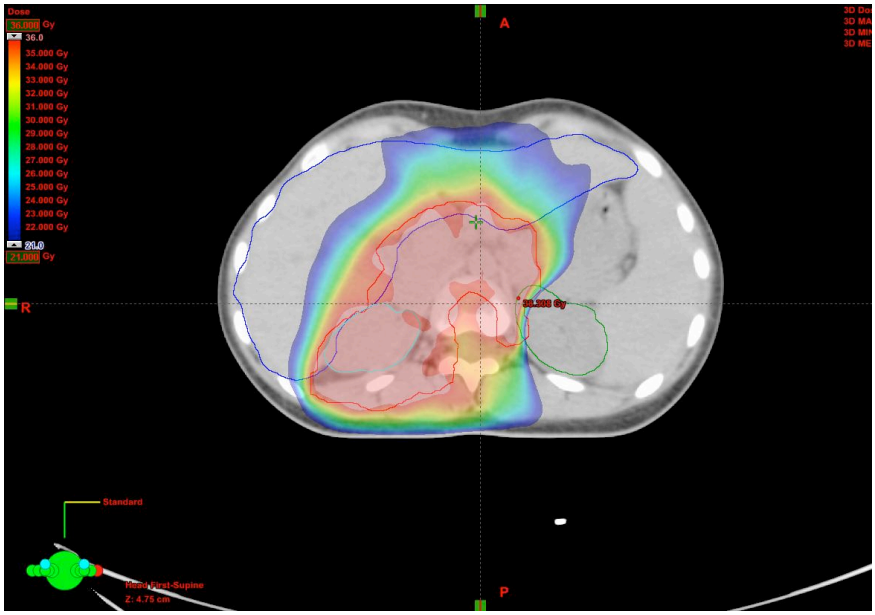
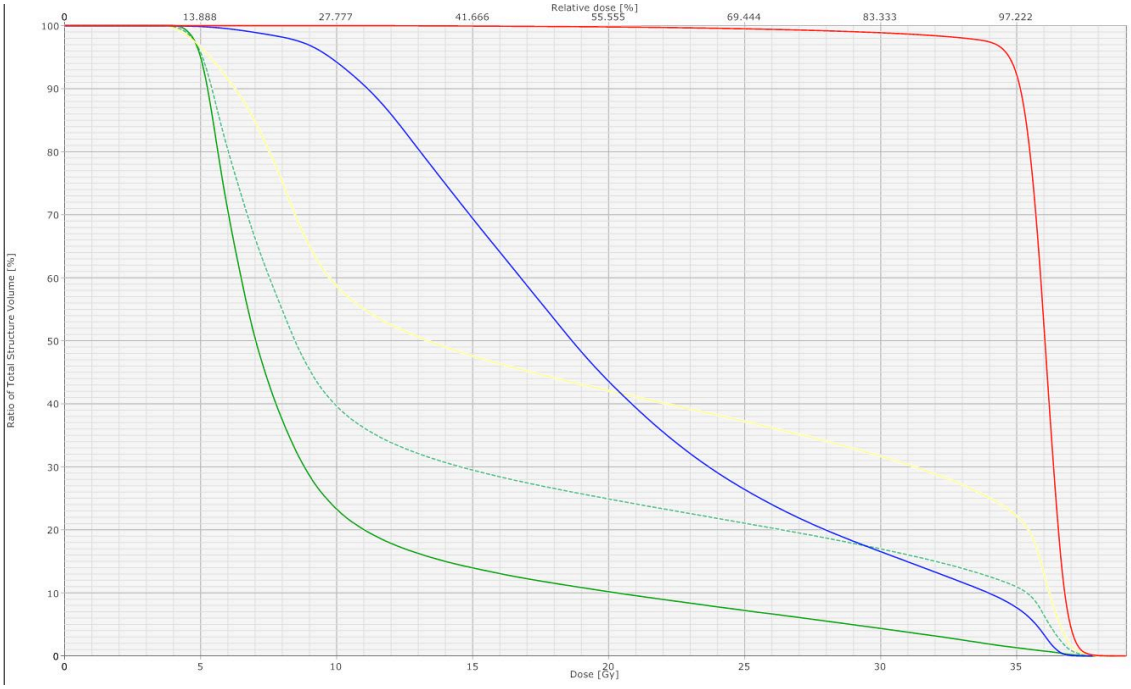


Figure 7.8B The DVH from the IMAT plan seen in Figure 7.8A. The PTV (red) is receiving 36Gy whilst keeping within the normal tissue constraints for the liver (blue), right kidney (yellow) and left kidney (green).

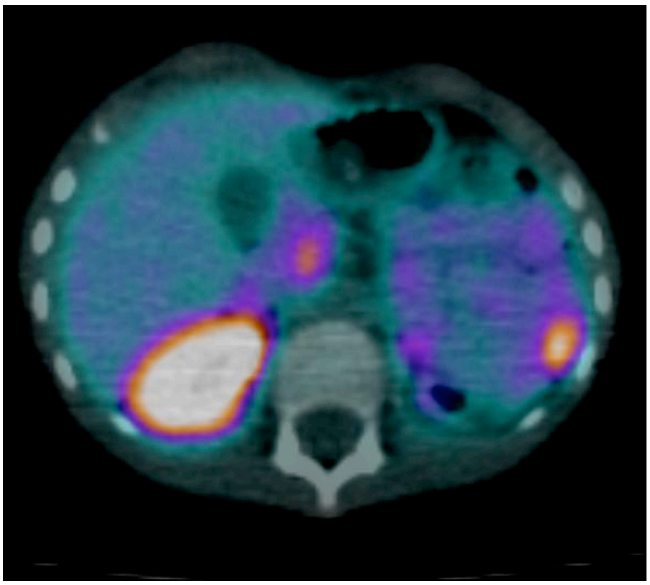


Clinical Case 3

7 year old boy with stage M neuroblastoma. Originally treated on the high-risk SIOPEN HR-NBL1 trial. Treated with multi-modality approach but required ^{131}I-mIBG and topotecan for poor response to initial therapy prior to high-dose myeloablative therapy and then received immunotherapy in Germany. Original surgical resection was very difficult and there was thought to be residual disease within the abdomen. Received standard abdominal radiotherapy using AP/PA fields in November 2010 as part of his multi-modality therapy. A two-phase technique was used (phase I 13.5Gy in 9 fractions and phase II 7.5Gy in 5 fractions) to stay within renal tolerance and this resulted in under-coverage of the PTV in phase II. The left kidney received the full 21Gy and 90% of the right kidney received 12-14Gy.

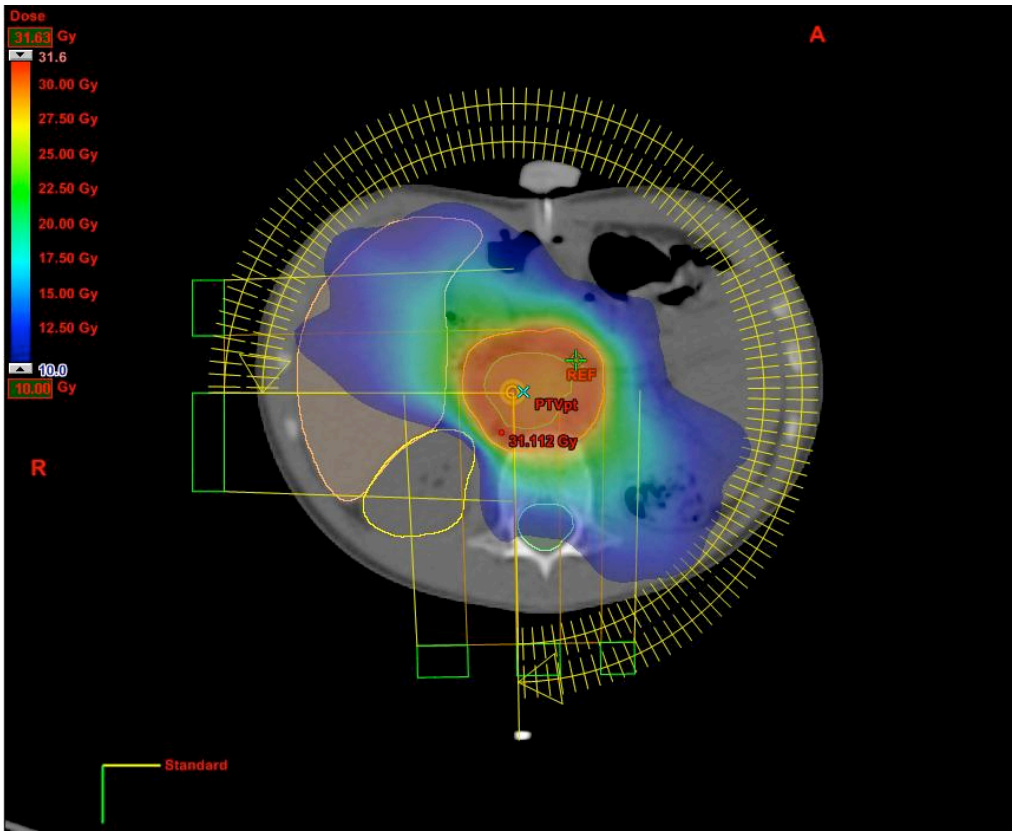
On follow-up imaging in Jan 2013, an ^{123}I-mIBG scan showed an increase in the size of local residual disease within the abdomen. A ^{68}Ga-DOTATATE PET/CT scan confirmed the uptake within the abdomen. As this was the only area of disease, it was decided to re-treat this area with external beam radiotherapy and an IMAT technique was chosen to reduce the dose to both kidneys. A dose of 30Gy in 20 fractions at 1.5Gy per fraction was used with the aim of maximizing disease control. An EDTA showed a satisfactory corrected creatinine clearance of 71mls/min/1.73m^2 and a DMSA scan showed differential kidney function with the right kidney contributing 68% of the total function and the left 32%.

Figure 7.9A The ^{68}Ga-DOTATATE PET/CT at the time of recurrence within the abdomen.



The CT planning scan was fused with the ^{68}Ga-DOTATATE PET/CT for biological target volume definition (BTV). The BTV was then modified to form a CTV and included the disease seen on the planning CT and a diagnostic CT. A margin of 0.7cm was added to the CTV to form a PTV.

Figure 7.9B The IMAT plan generated to treat the abdominal recurrence.



7.5 Discussion

The radiotherapy planning study presented here has been published by *Gains et al. (Future Oncology 2013)*. This is the largest study evaluating an IMRT technique in neuroblastoma and comparing it to the current standard conventional techniques employed over Europe to treat abdominal neuroblastoma. It has added to our knowledge of the possible benefits of IMAT in an unselected clinical series encompassing the heterogeneity of target volume and location seen in patients with abdominal neuroblastoma.

There have been two previous reports in the literature comparing IMRT techniques to conventional radiotherapy in neuroblastoma. Previous studies have had small patient numbers, often with mixed abdominal pathologies and radiation doses, which have not fully reflected the complexity of challenges faced by the clinical oncologist treating neuroblastoma.

A planning study by *Paulino et al.* compared conventional radiotherapy plans with fixed field IMRT plans in six children with neuroblastoma. They found an advantage with IMRT for midline tumours but found fixed field IMRT to be no better than conventional AP/PA fields for lateralised tumours because of an increased mean dose to the contralateral kidney (*Paulino 2006*). Our study however, has shown a benefit for IMAT in some patients with lateralised tumours who were protocol non-compliant with conventional radiotherapy.

Shaffer *et al.* reported a planning study comparing IMAT, fixed-field IMRT, 3D conformal radiotherapy (3D-CRT) and parallel-opposed beams in eight paediatric, retroperitoneally located tumours including five with neuroblastoma. Consistent with the findings by Paulino *et al.* they found an increase in mean contralateral kidney doses for lateralised tumours for the 3D-CRT, fixed-field IMRT and IMAT techniques (*Shaffer 2011*).

In this study, we found statistically significant improved conformity and dose distributions to the PTV with RapidArc™. In the *protocol compliant group*, which included in the majority lateralized tumours, we found no statistically significant change in the contralateral or ipsilateral kidney V15's, or ipsilateral mean kidney doses. There was a statistically significant increase in the contralateral mean kidney dose with RapidArc™, consistent with the two studies mentioned above.

With regards to liver doses for right-sided tumours, there was an advantage with RapidArc™ for patients in both groups although the numbers in each group were too small for statistical analysis (see Figure 7.4). Reducing the high-dose to the liver is important for those patients with veno-occlusive disease, a recognized complication of myeloablative therapy (*Horn 2002*).

The difference in anatomical location of tumours in the two different groups probably had a large influence on whether they were protocol complaint when treated conventionally or not. With lateralised tumours, it is easier to spare the contralateral kidney with the conventional treatment approach as opposed to large

midline tumours where both kidneys can fall within the treatment field. The greatest benefit from an IMAT technique therefore, is likely to be seen in large, midline abdominal neuroblastomas (see Figure 7.5).

In the *protocol non-compliant group,* the majority of tumors were midline and conventional planning had compromised dose, treated volume or both. There was an advantage for RapidArc^{TM} in this group, with eight of the ten cases being able to receive the full 21Gy, and in the other two cases, an improvement in the dose being received by the PTV compared to conventional planning.

When using conventional radiotherapy in children that may involve irradiating vertebrae, it has been recommended to include the whole vertebra within the radiation field to avoid partial irradiation which may lead to future growth asymmetry. In this study we compared the homogeneity of dose distribution across the vertebra (vertebral HI) and found this to be significantly improved with the IMAT compared to the conventional plans ($p<0.001$).

Advantages of RapidArc^{TM} over fixed field IMRT are reduced treatment times and a reduction in monitor units (*Cozzi 2008, Vieillot 2010, Wiezoreck 2011*). A reduction in treatment time may reduce the intra-fraction patient motion and a reduction in monitor units has important implications with regards to the development of secondary malignancies.

With the increased use of IMRT techniques it is essential to re-evaluate traditional treatment planning margins. With the increased conformity with new radiotherapy techniques and image–guided radiotherapy techniques it may be possible to reduce the treatment margins resulting in less normal tissue being irradiated. A recent report showed an effective reduction in setup margin for children with abdominal neuroblastoma based on daily cone beam CT rather than conventional weekly imaging (*Beltran 2010*).

As external beam radiotherapy improves local control in children with neuroblastoma, regardless of the extent of surgical resection, being able to deliver the full protocol dose to the PTV in more cases may reduce local recurrence. Dose escalation, respecting current normal tissue constraints, is difficult with current radiotherapy techniques. It is not known whether dose escalation beyond 21Gy may improve local control. Some groups have reported the routine use of up to 40Gy in patients with gross residual disease post surgical resection (*Simon 2006*). Dose escalation for residual disease is being assessed in the current Children's Oncology Group (COG-ANBL0532) high-risk neuroblastoma trial. Dose escalation is unlikely to be possible with conventional radiotherapy fields and therefore if a dose escalation question is to be asked in the next European high-risk study, techniques such as IMAT will need to be employed.

The possibility that IMRT may increase the risk of radiation-induced second cancers in children is important. The increased volume of normal tissue receiving a low dose from multiple beams and leakage of radiation from the linac head both contribute to

this risk. The second malignancy risk from IMRT in children was originally estimated to be double that of conventional treatment (*Hall 2006*) but more recent publications challenge this (*Schneider 2008*). We noted improved conformity of the high dose regions with the RapidArc technique. The non-PTV integral dose, acting as a proxy for the normal tissue receiving a low dose, was variable and not increased with RapidArc in all cases as would have been postulated (see Figure 7.6).

I am currently involved in an on-going project in collaboration with the paediatric radiotherapy group at looking at Addenbrookes Hospital, Cambridge. We are looking at three secondary malignancy risk models in our cohort of neuroblastoma patients discussed here in the planning study and a smaller cohort of patients from Cambridge treated with tomotherapy and comparing to conventional radiotherapy techniques.

This study highlights the fact that there are a number of children with abdominal neuroblastoma where the current SIOPEN high-risk neuroblastoma clinical trial protocol's standard radiotherapy technique, AP/PA fields, is unable to deliver the prescribed protocol dose. This protocol was written ten years ago, before IMRT and IMAT techniques were widely available. This study shows that for patients where conventional radiotherapy fields cannot deliver the full protocol dose, IMAT may be able to improve dose delivery and potentially impact on local control rates in neuroblastoma. However, it is not a class solution. There remain concerns including secondary carcinogenesis risk and an increased mean contralateral kidney dose in lateralised tumours with the IMAT technique. Any amendment to protocol

recommendations should be based on clear evidence of potential benefit, rather than an assumption that more modern techniques 'must' be better.

We have also shown that the technique we used in the planning study can be put into clinical practise. The three cases illustrated here would not have been able to receive the full protocol dose with conventional radiotherapy and we hope that this will have had an impact on local control for these patients.

Within the US there is an early phase trial based at St Judes examining fixed field IMRT for neuroblastoma. The same group from St Judes have recently reported on 20 children with high-risk neuroblastoma treated with IMRT. Radiotherapy doses varied depending on the degree of surgical resection – 23.4Gy, 30Gy, 30.6Gy or 36Gy. With a median follow-up of 2.2 years, there were no primary site in-field or loco-regional failures (*Pai Panandiker 2013*).

Before theoretically attractive innovative therapies are introduced into routine clinical practice, it is essential that they are scientifically evaluated in clinical trials. A clinical trial within the UK has been proposed and supported form the relevant NCRI clinical studies groups and has now received support from CRUK via CTAAC.

This study addresses a possible solution for the real clinical problem that a significant number of patients with neuroblastoma cannot receive adequate radiotherapy with conventional radiotherapy techniques. We have demonstrated that IMAT can improve dose distributions and the number of patients receiving the full protocol

dose. The real value of IMAT in neuroblastoma will be investigated in a prospective clinical trial which I have designed with colleagues.

Chapter 8

Discussion

The overall survival for high-risk neuroblastoma remains under 40% despite therapeutic advances and intensification of therapy. Radiotherapy, both external beam and molecular radiotherapy, form an essential part of the multi-modality treatment of this disease. This work has shown several ways of optimising existing therapies and developing new potential radiotherapeutic techniques and treatments for the management of high-risk neuroblastoma.

Molecular radiotherapy is an attractive systemic treatment option for a disease such as high-risk neuroblastoma which is often widely metastatic. Historically, the only molecular radiotherapy agent in use for neuroblastoma was ^{131}I-mIBG targeting the noradrenaline transporter molecule. Unfortunately, despite its widespread use since the mid 1980's, the systematic review, presented in Chapter 4, revealed that not a single randomised trial of ^{131}I-mIBG in neuroblastoma has been performed (*Wilson 2013*). The studies have been Phase I, Phase II, or case series and they have all used a variety of schedules and administered activities in a highly heterogenous population. The studies reflect the fact that neuroblastoma is a relatively rare disease and ^{131}I-mIBG delivery has been only possible in select centres with appropriate facilities to deliver molecular radiotherapy to children and young people. Despite this, the systematic review analysis revealed an overall mean tumour response of 32% in the relapsed and refractory setting which is highly promising compared to conventional chemotherapy regimes in this setting.

The systematic review of ^{131}I-mIBG therapy will be valuable in aiding future areas of research. It is essential that ^{131}I-mIBG is evaluated in prospective, randomised clinical trials. Both within Europe and the United States, ^{131}I-mIBG is to be used in planned prospective trials for poor responders to initial induction therapy.

This work has shown that neuroblastomas also express somatostatin receptor type 2 and therefore give an alternative molecular radiotherapy target, radiolabelled somatostatin analogues, for both the imaging and treatment of neuroblastoma. The feasibility of performing ^{68}Ga-DOTATATE PET/CT for disease extent assessment and selection of patients for treatment with ^{177}Lu-DOTATATE has been shown. In a small pilot study of patients with relapsed or refractory neuroblastoma it was shown for the first time that ^{177}Lu-DOTATATE therapy is a potential treatment option and was shown to be safe in the short follow up of the study (*Gains 2011*). The formal evaluation of safety and response to ^{177}Lu-DOTATATE in relapsed and refractory neuroblastoma is currently underway in a phase II trial – the LuDO trial, which has been developed as a result of the work presented here.

This collection of work has shown that the expression of NAT and SSTR2 in neuroblastomas is not homogenous both on functional imaging (see Chapter 2) and by immunohistochemistry of archived neuroblastoma tissue (see Chapter 3). This has important implications for the diagnosis, staging, response assessment and treatment of high-risk neuroblastoma. It suggests that somatostatin receptor imaging may have a significant role to play alongside mIBG.

We have changed our molecular radiotherapy practise. Prior to this work on radiolabelled somatostatin analogues the only agent used for molecular radiotherapy in neuroblastoma was ^{131}I-mIBG. This work has shown that imaging with radiolabelled somatostatin analogues provide additional information from functional imaging compared to ^{123}I-mIBG alone. The use of ^{177}Lutetium DOTATATE may provide a better molecular radiotherapy therapeutic option due to the distribution of disease in some patients with neuroblastoma. The results of the phase II, LuDO study are awaited to assess response rate and toxicity but having seen such heterogeneity in distribution, a combination of both agents may be a potential future therapeutic option.

Over the past decade, investigators have looked to increase the response to ^{131}I-mIBG by using higher administered activities, requiring stem cell support and the use of radiosensitisers such as topotecan and irinotecan (*Gaze 2005, Dubois* 2012). Recent preclinical studies have shown that Vorinostat (an inhibitor of HDAC) can increase the expression of the norepinephrine transporter in neuroblastoma in vitro and in vivo which could have potential for enhancing ^{131}I-mIBG therapy (*More 2011*). The combination of vorinostat and ^{131}I-mIBG is now being evaluated in a phase I study. Pre-clinical evidence has shown additional benefit for the use of PARP inhibitors, which also act as radiation sensitisers. They have been shown in laboratory models to have synergy with ^{131}I-mIBG molecular radiotherapy (*McCluskey 2012*). Clinical evaluation of PARP inhibitors in combination with ^{131}I-mIBG is awaited.

As well as the above mentioned strategies to improve the response rate to ^{131}I-mIBG there are several potential reasons why patients with neuroblastoma fail to respond to molecular radiotherapy which must be considered for future development and strategies. Although neuroblastoma is commonly described as a radiosensitive tumour there is likely to be a range of radioresponsiveness, especially in heavily pre-treated patients. Neuroblastoma is a very heterogenous disease and the laboratory data available is on a limited umber of neuroblastoma cell lines and therefore, not all neuroblastomas may be intrinsically radiosensitive.

The findings of non-homogenous distribution of the NAT and SSTR2 receptor targets could result in a non-uniform distribution of radiation dose to the tumour cells. Molecular radiotherapy uptake is likely to be heterogenous and the poor vascularity of tumours and central necrosis of larger lesions can add to the variable distribution. Some of the heterogeneity of uptake will be overcome by the potential cross fire effect between adjacent cells as well as the bystander effect (*Boyd 2006, Gow 2013*).

Tumour hypoxia may be another reason for inadequate response to radiotherapy. There have been no studies looking at neuroblastoma tumour hypoxia on functional imaging but tumour hypoxia has been demonstrated on histopathological specimens by immunohistochemistry using the HIF-1α antibody. High levels of HIF-1α were associated with aggressive clinical behaviour of neuroblastoma (*Dungwa 2012*).

The physical properties of radioisotope are important for targeting the receptor positive cells but also for targeting cells not expressing the receptor. When selecting

radioisotopes for therapy rather than imaging it is important to take in to account the type and energy of the emitted radiation, the distance over which the energy is deposited, as well as the physical half-life of the radioisotope.

In the vast majority of studies on molecular radiotherapy within the literature there has been no dosimetry performed so that we do not know what range of tumour doses patients are receiving. Historically dosimetry has been difficult to perform and came with inherent problems with regards to accuracy. If molecular radiotherapy is to earn its role in the management of high-risk neuroblastoma dosimetry will need to be performed. It is essential to know the absorbed dose to the tumour as well as critical organs at risk as we would do in every day external beam radiotherapy practise. Within the phase II LuDO trial, developed as a result of this work, we will measure tumour dosimetry and correlate this with response as well as looking at organ dosimetry.

The role of ^{18}F-FDG-PET is unclear in neuroblastoma and there is little available data in the published literature. The additional benefit of ^{18}F-FDG-PET for assessing response to ^{131}I-mIBG therapy was explored in this work. For this particular indication, ^{18}F-FDG PET/CT is unlikely to be a valuable tool in assessing relapsed or refractory disease in high-risk neuroblastoma patients due to the low-grade uptake seen and difficulty in interpreting scans in heavily pre-treated patient and those who have received recent chemotherapy or G-CSF. The role of ^{18}F-FDG PET/CT is more likely to be of benefit in assessing early response to induction chemotherapy following initial diagnosis but this requires prospective examination. Other,

alternative functional imaging techniques are being examined around the world such as ^{18}F-dopa PET/CT (*Piccardo 2012*).

The development of new functional imaging modalities for the management of neuroblastoma is an exciting area of research. However, these newer techniques are unlikely to be widely available which does limits their use, reproducibility and inclusion in large prospective clinical trials for evaluation. ^{123}I-mIBG is therefore likely to remain, for the foreseeable future, the gold-standard imaging modality for the diagnosis, staging and response assessment of metastatic neuroblastoma and we await the evaluation of ^{124}I-mIBG PET/CT which is now in an early phase clinical trial.

With regards to local control in neuroblastoma, most current high-risk neuroblastoma protocols from around the world include external beam radiotherapy to the primary tumour. There is evidence to support that using external beam radiotherapy improves local control and some small studies that suggest that local control in high-risk neuroblastoma may impact on overall survival for these patients. We have seen in this study that almost 50% of the patients treated historically with external beam radiotherapy had a compromise on their tumour volume coverage due to an inability to safely deliver the protocol dose within organ at risk dose constraints. Within the planning study performed, dose delivery to the target volume was significantly improved with an IMAT technique compared to traditional parallel-opposed radiotherapy techniques. There were also significant advantages in terms of doses to the liver with the IMAT technique for right-sided tumours (*Gains 2012*).

There have been concerns over adopting IMRT techniques for paediatric tumours because of the low-dose volume spread and the potential risk of secondary malignancies. It is right that theses techniques are not universally adopted in paediatrics as in the adult oncology population and rather they should be investigated prospectively in clinical trials as is proposed here. The evaluation of secondary malignancy risk comparing IMAT and conventional radiotherapy in abdominal neuroblastoma is an on going collaboration with another UK paediatric radiotherapy centre.

If the next European high-risk neuroblastoma study wants to ask a dose escalation of local radiotherapy question, it is unlikely to be possible without the introduction of new radiotherapeutic techniques as too many patients receive an inadequate dose or tumour coverage with conventional techniques already.

There is retrospective quality assurance of radiotherapy in the current SIOPEN high-risk neuroblastoma study but it will be essential that this becomes prospective if these new techniques are employed over Europe (*Gaze 2010, Gaze 2013*). Poor compliance with radiotherapy protocols within clinical trials has been found to have an adverse affect on clinical outcomes (*Ohri 2013*).

Another way of potentially sparing the dose to normal tissues is to examine the role of proton beam therapy. Protons are heavy, positively charged particles that stop within tissues depositing 90% of their energy (the Bragg peak). Following the 'Bragg peak' there is a sharp fall off of dose so that even tumours next to critical organs at

risk can often be treated. With regards to paediatric patients, the greatest potential for protons comes from the reduced likelihood of long term toxicities and complications of treatment resulting from a reduced dose to normal tissues and a potential reduction in secondary malignancies. There are three reports on the use of proton therapy for the treatment of neuroblastoma in the literature (*Hillbrand 2008, Hattanhadi 2011, Hill-Kayser 2013*).

In conclusion, this work has shown several ways in which radiotherapeutic techniques, both external beam and molecular radiotherapy, could be optimised with the aim of improving the outcome for patients with high-risk neuroblastoma. As a result of this work we have opened the phase II trial of ^{177}Lutetium DOTATATE in relapsed/refractory high-risk neuroblastoma and have secured CTAAC funding via CRUK for a phase II trial of IMAT in high-risk neuroblastoma.

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